(54) Title: COMPOUNDS THAT BIND HER2

(57) Abstract: The invention provides novel compounds which bind to the human erbB2 gene product (ErbB2, also known as HER2, or c-ErbB-2). In particular aspects, the invention provides for the treatment of disorders characterized by the overexpression of ErbB2 utilizing the novel compounds of the invention. The invention also provides pharmaceutical compositions comprising the novel compounds as well as for their use in research, diagnostic. therapeutic, and prophylactic methods.

COMPOUNDS THAT BIND HER2

Background of the Invention

Field of the Invention

This invention relates to novel compounds which bind to the human erbB2
5   gene product (ErbB2, also known as HER2, or c-ErbB-2). In particular aspects,
the invention relates to the treatment of disorders characterized by the
expression of ErbB2 utilizing the novel compounds of the invention. The
invention also relates to compositions, such as pharmaceutical compositions,
comprising the novel compounds, as well as their use in research, diagnostic,
10   therapeutic, and prophylactic methods.

Description of Related Disclosures

Phage display provides a means for generating peptides and protein
variants through randomization of specific amino acid residues within a
template sequence or by generation of naive peptide libraries (Lowman, H.
15   (1998) Methods Mol. Biol. 87:249-264; Lowman (1997) Annu. Rev. Biophys. Biomol.
Struct. 26:401-424). Identification and isolation of displayed proteins or
peptides that bind a predetermined target molecule can be achieved through
enrichment of displaying phage over non-binding or weakly binding variants on
immobilized target molecules (Lowman, H. (1989), supra). Successive rounds of
20   mutagenesis and selection can yield peptide ligands or protein variants with
high affinity for cellular receptors (Lowman, H. (1998), supra). The technique
has been used to identify peptide motifs that home to, for example, a cellular
target (Arap et al., (1998) Science 279:377-380), or to generate affinity
improved or matured peptide ligands from native protein binding ligands (Lowman
25   et al., (1991) Biochemistry 30: 10832-10838). Examples of affinity or
specificity improved proteins include human growth hormone, zinc fingers,
protease inhibitors, atrial natriuretic peptides, and antibodies (Wells, J. and
Lowman H. (1992) Curr. Opin. Struct. Biol. 2:597-604; Clackson, T. and Wells,
J. (1994) Trends Biotechnol. 12:173-184; Lowman et al., (1991) Biochemistry
30   30(10):832-838; Lowman et al. and Wells J. (1993) J. Mol. Biol. 234:564-578;
Dennis M. and Lazarus R. (1994) J Biol. Chem. 269(22):137-144).

Utilizing *in vivo* phage selection, phage display has been used to
identify and isolate peptides capable of mediating selective localization to
various organs such as brain and kidney (Pasqualini and Ruoslohti (1996) Nature
35   380:364-366) as well as to identify peptides that home to particular tumor
types bearing $\alpha_v\beta_3$ or $\alpha_v\beta_5$ integrins (Arap et al., (1998) Science 279:377-380).
U.S. Patent 5,627,263 describes peptides that are recognized by and selectively
bind the $\alpha_5\beta_1$ integrin. Using structurally constrained peptide libraries
generated by monovalent phage display, 14 amino acid peptides that specifically
40   bind to insulin-like growth factor 1 binding proteins (IGFBPs) have been
isolated (Lowman et al., (1998) Biochemistry, 37:8870-8878). The peptides
contain a helix structure and bind IGFBPs *in vitro* liberating insulin like
growth factor-α (IGF-1) activity (Lowman et al., (1998) supra).

Particular cellular receptors and their ligands, especially those implicated in the pathogenesis of various human malignancies, have recently been the focus of much attention in the scientific community as novel protein based therapeutics enter the clinic. For example, it has been found that the human erbB2 gene (also known as her2, or c-erbB-2), which encodes a 185-kd transmembrane glycoprotein receptor ($p185^{HER2}$) related to the epidermal growth factor receptor (EGFR), is overexpressed in about 25% to 30% of human breast cancer (Slamon et al., (1987) Science 235:177-182; Slamon et al., (1989) Science 244:707-712). Several lines of evidence support a direct role for ErbB2 in the pathogenesis and clinical aggressiveness of ErbB2-overexpressing tumors. The introduction of erbB2 into non-neoplastic cells has been shown to cause their malignant transformation (Hudziak et al., (1987) Proc. Natl. Acad. Sci. USA 84:7159-7163; DiFiore et al., (1987) Science 237:78-182). Transgenic mice that express HER2 were found to develop mammary tumors (Guy et al., (1992) Proc. Natl. Acad. Sci. USA 89:10578-10582).

ErbB2 overexpression is commonly regarded as a predictor of a poor prognosis, especially in patients with primary disease that involves axillary lymph nodes (Slamon et al., (1987) and (1989), supra; Ravdin and Chamness, (1995) Gene 159:19-27; and Hynes and Stern, (1994) Biochim. Biophys. Acta 1198:165-184). Overexpression has also been linked to sensitivity and/or resistance to hormone therapy and chemotherapeutic regimens, including CMF (cyclophosphamide, methotrexate, and fluoruracil) and anthracyclines (Baselga et al., (1997) Oncology 11(3 Suppl 1):43-48).

A recombinant humanized anti-ErbB2 monoclonal antibody (a humanized version of the murine anti-ErbB2 antibody 4D5, referred to as rhuMAb HER2 or HERCEPTIN®) is therapeutically active in patients with ErbB2-overexpressing metastatic breast cancers that have previously received extensive anti-cancer therapy (Baselga et al., (1996) J. Clin. Oncol. 14:737-744).

Summary of the Invention

The present invention provides compounds which bind to the human erbB2 gene product (ErbB2). The compounds of the present invention (referred to as peptide ligands herein) are, for example, peptides or peptide derivatives such as peptide mimetics and peptide analogs. Preferred peptide ligands include linear and cyclic peptides, preferably cyclic peptide compounds comprising the following core formula:

-Cys-Xaa-Xaa-Xaa-Xaa-Cys-

and preferably those having the general formula:

Cys-Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Cys-Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Cys-Cys-Xaa-Xaa-Xaa-Cys-Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Cys (SEQ ID NO:70)

or the general formula:

Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Cys-Xaa-Xaa-Xaa-Xaa-Cys-Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Xaa, including dimers and other combinations of the foregoing general structures. Preferred among the peptide ligands of the invention are peptides comprising the core formula:

-Cys-Cys/Ile-Gly-Pro-Gly-Cys- (SEQ ID NO:71)

Specific examples cf such compounds include cyclic peptides of between about 10 and 60 amino acid residues and optionally including N- or C-terminal modifications or both, such as esters, amides, salts and other derivatives thereof. According to preferred aspects of the invention, the compounds are preferably non-naturally occurring amino acid sequences that bind ErbB2. Preferably the compound is a non-naturally occurring amino acid sequence of between about 5 and about 50 amino acid residues. Preferred compounds are cyclic peptides having theforegoing general formulae and having of between about 20 and about 30 amino acid residues and include dimers and other combinations thereof.

In particular aspects the invention is directed to combinations of peptide ligands with other peptide ligands or with other proteins, especially serum proteins or peptides. The combinations are prepared with various objectives in mind, including; increasing the affinity or avidity of the peptide ligand for its target molecule, as for example, by use of various multimerization domains; increasing the stability of the peptide ligand or facilitating its recovery and purification, as for example by expressing the peptide ligand as a Z protein fusion; improving the therapeutic efficacy of the peptide ligand in aspects of the invention involving *in vivo* use of the peptide ligand, by for example, increasing or decreasing the serum half life, by for example, fusing the peptide ligand to a plasma protein such as serum albumin, an immunoglobulin, apolipoproteins or transferrin (such fusion being made conveniently in recombinant host cells or by the use of bifunctional crosslinking agents)and introducing additional functionalities, as for example, those of a functional Fc domain or cytotoxic or enzyme moiety.

According to a preferred aspect of the present invention the compound of the invention is linked to a multimerization domain. The multimerization domain is preferably an immunoglobulin sequence or, for example, a leucine zipper sequence. According to this aspect of the invention the immunoglobulin sequence is preferably an immunoglobulin constant region sequence and especially the constant region of an immunoglobulin heavy chain. According to preferred aspects of the invention, the multimerization domain pairs with one or more companion multimerization domains to provide homo- and hetero-multimer compositions. Preferred according to this aspect of the present invention are homo- and hetero-multimers, especially homo- and heterodimers wherein the multimerization domains are immunoglobulin heavy chain constant regions which pair to provide functional immunoglobulin Fc domains. Therefore, according to preferred aspects, the invention provides a hybrid molecule comprising a compound of the invention which functions to target an ErbB2 bearing cell type and functional immunoglobulin Fc domain possessing an effector function associated with a functional immunoglobulin Fc domain.

Hybrid molecules according to this aspect of the invention optionally include a further functional moiety such as an enzyme moiety or a cytotoxic

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moiety. For example, the additional functional domain may be an enzyme covalently linked to a hybrid molecule and capable of acting on a prodrug in such a way as to convert the prodrug to its more active form. The optional functional domain, according to certain preferred aspects of the invention, may

5   be a cytotoxic agent linked by, for example, covalent attachment, to a hybrid molecule. Preferred cytotoxic agents include, for example, chemotherapeutic agents, toxins and radioactive isotopes. The compounds including the hybrid molecules and compositions comprising them are used in binding or detecting the human erbB2 gene product and optionally delivering a functional moiety such as

10   an enzyme or cytotoxic drug to an ErbB2 bearing cell type.

In one embodiment, the compound of the present invention, such as the hybrid molecule described above, is a polypeptide and the invention encompasses a composition of matter comprising an isolated nucleic acid, preferably DNA, encoding the polypeptide of the invention. According to this aspect, the

15   invention further comprises an expression control sequence operably linked to the DNA molecule, an expression vector, preferably a plasmid, comprising the DNA molecule, where the control sequence is recognized by a host cell transformed with the vector, and a host cell transformed with the vector. According to preferred aspects the nucleic acid encodes a hybrid molecule

20   comprising a peptide compound of the invention and an immunoglobulin constant region domain sequence. The nucleic acid molecule according to this aspect of the present invention encodes a hybrid molecule and the nucleic acid encoding the peptide compound of the invention is operably linked to (in the sense that the DNA sequences are contiguous and in reading frame) the immunoglobulin

25   domain sequence. Optionally the DNA sequences may be linked through a nucleic acid sequence encoding an optional linker domain amino acid sequence.

The compositions of the present invention may be made by a process which includes the steps of isolating or synthesizing nucleic acid sequences encoding any of the amino acid sequences of the invention, ligating the nucleic acid

30   sequence into a suitable expression vector capable of expressing the nucleic acid sequence in a suitable host, transforming the host with the expression vector into which the nucleic acid sequence has been ligated, and culturing the host under conditions suitable for expression of the nucleic acid sequence, whereby the protein encoded by the selected nucleic acid sequence is expressed

35   by the host. Preferably, the polypeptide is then recovered from the host cell culture. In this process, the ligating step may further contemplate ligating the nucleic acid into a suitable expression vector such that the nucleic acid is operably linked to a suitable secretory signal, whereby the amino acid sequence is secreted by the host.

40   The invention includes compositions, including pharmaceutical compositions, comprising the compounds of the invention, especially for the detection and treatment of diseases or disorders associated with erbB2 gene expression. Provided are, for example, kits and articles of manufacture for

the detection and treatment of cancer. Kits and articles of manufacture preferably include:

            (a)   a container;

            (b)   a label on or associated with said container; and

5   (c)   a composition comprising a compound of the present invention contained within said container wherein, according to certain embodiments, the composition is effective for treating a disease or disorder associated with erbB2 gene expression, such as cancer. Preferably, the label on said container indicates that the composition can be used for treating cancer, and the

10   compound in said composition comprises a compound as described herein which binds to the human erbB2 gene product. The kits optionally include accessory components such as a second container comprising a pharmaceutically-acceptable buffer and instructions for using the composition to treat a disorder.

          Also disclosed are methods useful in the treatment of various diseases

15   and disorders, such as cancer, especially those characterized by the involvement of ErbB2. Therefore, the invention provides a method of treating an ErbB2 mediated or associated disease or disorder in a host in need thereof comprising administering to the host a therapeutically effective amount of a compound of the invention. The present invention further provides various

20   dosage forms of the compounds of the present invention, including but not limited to, those suitable for parenteral, oral, rectal and pulmonary administration of a compound. In preferred aspects of the present invention, a therapeutic dosage form is provided suitable for intravenous delivery and the invention provides for the treatment of diseases or disorders involving a ErbB2

25   mediated or associated process or event such as cancer.

Brief Description of the Drawings

         Figure 1 is a schematic representation of a native IgG and fragments thereof resulting from papain and pepsin digest. Disulfide bonds are represented by -S-S- between, for example, the CH1 and CL domains. In the

30   figure, V is variable domain; C is constant domain; L stands for light chain and H stands for heavy chain.

         Figure 2A depicts alignments of native IgG Fc region amino acid sequences. Shown are human (hum) IgG Fc region sequences, humIgG1 (non-A and A allotypes) (SEQ ID NOs:72 and 73 , respectively), humIgG2 (SEQ ID NO:74),

35   humIgG3 (SEQ ID NO:75) and humIgG4 (SEQ ID NO:76), are shown. The human IgG1 sequence is the non-A allotype, and differences between this sequence and the A allotype (at positions 356 and 358; EU numbering system) are shown below the human IgG1 sequence. Also shown are native murine (mur) IgG Fc region sequences, murIgG1 (SEQ ID NO:77), murIgG2A (SEQ ID NO:78), murIgG2B (SEQ ID

40   NO:79) and murIgG3 (SEQ ID NO:80), are also shown. Figure 2B shows the percent identity among the Fc region sequences of Figure 2A.

         Figure 3 shows the alignments of native human IgG Fc region sequences, humIgG1 (non-A and A allotypes) (SEQ ID NOs:72 and 73), humIgG2 (SEQ ID NO:74),

humIgG3 (SEQ ID NO:75) and humIgG4 (SEQ ID NO:76) with differences between the sequences marked with asterisks.

Figure 4 shows the enrichment of polyvalent peptide phage that bind the HER2 extracellular domain (ECD). The ratio of phage eluted from a well coated with HER2 versus a well coated with BSA is shown for rounds 3 and 4 of selection.

Figure 5 shows the results of a phage binding assay. Phage clones 1.1, 5.1 and 7.1 were found to bind immobilized HER2-ECD, but not BSA or closely related HER3 or HER4 indicating they specifically recognized HER2-ECD.

Figure 6 shows EC50 determination for peptide 1.1.FIZb binding immobilized HER2.

Figure 7 shows the results of a competition assay for synthetic peptides HER201 and HER212 as well as recombinant peptides 1.1.FI-Z and 7.1c-Z with peptide 1.1.FI-Zb for binding to immobilized HER2-ECD.

Figures 8A-C. Figure 8A shows the 1.1.FI-Z (SEQ ID NO:81) preparation (S) purified using a Superdex 75 into monomer (M) and dimer (D) containing fractions. Figure 8B shows an SDS PAGE analysis of starting material and fractions from the SUPERDEX™ 75 column for peptide 1.1.FI-Z. Figure 8C shows HER2 binding activity coinciding with dimer fractions of 1.1.FI-Z when assayed using a competition assay.

Figures 9A-C. Figure 9A shows the $(1.1FI)_2$-Z (SEQ ID NO:82) (1.1FI repeated twice and fused to the Z domain of protein A) preparation purified using a SUPERDEX™ 75 to monomer and dimer containing fractions. Figure 9B shows and SDS PAGE analysis of fractions from the SUPERDEX™ column for peptide $(1.1FI)_2$-Z. Figure 9C shows HER2 binding activity coinciding with monomer fractions of $(1.1FI)_2$-Z when assayed using a competition assay.

Figures 10A and B. Figures 10A and 10B show that peptides HER201 and 7.1c-Z block the binding of Class 1 and Class 7 phage suggesting that both classes of peptides bind to the same site on HER2.

Figure 11 shows examples of Class 1 and Class 7 sequences revealing a core homology region between the two classes of peptides. Sequence 7.1c (SEQ ID NO:83), 1.1.2 (SEQ ID NO:84), 1.1.FC (SEQ ID NO:85), 1.1.FA (SEQ ID NO:86), 1.1.FB (SEQ ID NO:87), 1.1.FH (SEQ ID NO:88), 1.1.CF (SEQ ID NO:89).

Figure 12 shows the results of a competition assay of Class 1 and Class 7 peptides with known monoclonal antibodies directed against HER2 for binding HER2-ECD. The peptides do not compete with the monoclonal antibodies for binding HER2-ECD suggesting that the peptides bind a HER2-ECD site distinct from that recognized by known monoclonal antibodies.

Figure 13 shows the results of a binding assay conducted using peptide 1.1.FI-Zb and cells either expressing HER2 at different levels (MDA 361 [$4x10^5$ receptors/cell]) and BT 474 ([$3x10^6$ receptors/cell]) or cells not expressing HER2 (BaF3). 1.1.FI-Zb was found to bind cells proportional to the number of HER2 receptors expressed on the cell and concentration of 1.1.FI-Zb peptide.

Figure 14 shows the results of SDS PAGE analysis under oxidizing and reducing conditions of peptide 1.1.FI fused through a linker to the hinge, CH1 and CH2 domains of human IgG1 (1.1FI-Fc). The molecular weight shift under the two conditions suggests dimerization of the Ig constant regions and the formation of a Fc domain.

Figure 15 shows the deduced peptide sequences from clones in libraries 1.1 and 7.1. Sequence 1.1 (SEQ ID NO:57); 1.1.8 (SEQ ID NO:58); 1.1.9 (SEQ ID NO:59); 1.1.10 (SEQ ID NO:60); 1.1.1 (SEQ ID NO:61); 1.1.2 (SEQ ID NO:62); 1.1.6 (SEQ ID NO:63); 1.1.11 (SEQ ID NO:64); 1.1.3 (SEQ ID NO:65); 1.1.4 (SEQ ID NO:66); 1.1.5 (SEQ ID NO:67); 1.1.7 (SEQ ID NO:68); Consensus (SEQ ID NO:69); 7.1 (SEQ ID NO:45); 7.1.1 (SEQ ID NO:46); 7.1.9 (SEQ ID NO:47); 7.1.6 (SEQ ID NO:48); 7.1.5 (SEQ ID NO:49); 7.1.3 (SEQ ID NO:50); 7.1.4 (SEQ ID NO:51); 7.1.2 (SEQ ID NO:52); 7.1.7 (SEQ ID NO:53); 7.1.8 (SEQ ID NO:54); 7.1.11 (SEQ ID NO:55); Consensus (SEQ ID NO:56).

Figure 16 shows the sequences from random clones for each library by the fourth round of selection. Sequence 1.1 (SEQ ID NO:57); Consensus (SEQ ID NO:58); 1.1.FC (SEQ ID NO:30); 1.1.FN (SEQ ID NO:31); 1.1.FA (SEQ ID NO:1); 1.1.FM (SEQ ID NO:2); 1.1.FE (SEQ ID NO:3); 1.1.FH (SEQ ID NO:29); 1.1.FB (SEQ ID NO:12); 1.1.FF (SEQ ID NO:13); 1.1.FI (SEQ ID NO:14); 1.1.FK (SEQ ID NO:15); 1.1.FD (SEQ ID NO:16); 1.1.FQ (SEQ ID NO:17); 1.1.FS (SEQ ID NO:18); 1.1.FX (SEQ ID NO:9); 1.1.FY (SEQ ID NO:10); 1.1.FZ (SEQ ID NO:11); 1.1.NC (SEQ ID NO:4); 1.1.NB (SEQ ID NO:5); 1.1.NM (SEQ ID NO:6); 1.1.NQ (SEQ ID NO:7); 1.1.NA (SEQ ID NO:19); 1.1.NG (SEQ ID NO:20); 1.1.NH (SEQ ID NO:21); 1.1.NE (SEQ ID NO:22); 1.1.NJ (SEQ ID NO:23); 1.1.NF (SEQ ID NO:24); 1.1.NK (SEQ ID NO:25); 1.1.NR (SEQ ID NO:26); 1.1.CF (SEQ ID NO:28); 1.1.CA (SEQ ID NO:32); 1.1.CB (SEQ ID NO:33); 1.1.CC (SEQ ID NO:34); 1.1.CD (SEQ ID NO:35); 1.1.CE (SEQ ID NO:36); 1.1.CG (SEQ ID NO:37); 1.1.CH (SEQ ID NO:38); 1.1.CI (SEQ ID NO:39); 1.1.CJ (SEQ ID NO:40); 1.1.CL (SEQ ID NO:41); 1.1.CM (SEQ ID NO:42); 1.1.CP (SEQ ID NO:43).

Figure 17 shows the results of a competition assay of 1.1FI-Fc and 1.1FI-Z with peptide 1.1FI-Zb for binding to immobilized HER2-ECD.

Detailed Description of the Preferred Embodiments

Definitions

The terms "HER2", "ErbB2" "c-Erb-B2" are used interchangeably. Unless indicated otherwise, the terms "ErbB2" "c-Erb-B2" and "HER2" when used herein refer to the human protein and "her2", "erbB2" and "c-erb-B2" refer to the human gene. The human erbB2 gene and ErbB2 protein are described in, for example, Semba et al., (1985) PNAS (USA) 82:6497-6501 and Yamamoto et al. (1986) Nature 319:230-234 (Genebank accession number X03363). ErbB2 comprises four domains (Domains 1-4).

The term "peptide ligand" within the context of the present invention is meant to refer to amino acid sequences, regardless of their origin, that function to bind ErbB2. Peptide ligands within the context of the present invention are generally constrained (that is, having some element of structure

as, for example, the presence of amino acids which initiate a β turn or β pleated sheet, or for example, cyclized by the presence of disulfide bonded Cys residues) or unconstrained (e.g., linear) amino acid sequences of less than about 50 amino acid residues, and preferably less than about 40 amino acid

5   residues. Of the peptide ligands less than about 40 amino acid residues, preferred are the peptide ligands of between about 10 and about 30 amino acid residues and especially the peptide ligands of about 20 to about 30 amino acid residues. However, upon reading the instant disclosure, the skilled artisan will recognize that it is not the length of a particular peptide ligand but its

10   ability to bind ErbB2 that distinguishes the peptide ligand of the present invention. Therefore, peptide ligands of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30 amino acid residues, for example, are equally likely to be peptide ligands within the context of the present invention.

15   A peptide ligand of the present invention will bind ErbB2 with sufficient affinity and specificity if the peptide ligand "homes" to, "binds" or "targets" ErbB2, such as an ErbB2 bearing cell, in vitro and preferably in vivo (see, for example, the use of the term "homes to," "homing," and "targets" in Pasqualini and Ruoslahti (1996) Nature, 380:364-366 and Arap et al., (1998) Science

20   279:377-380). In general, the peptide ligand will bind ErbB2 with an affinity of less than about 1 μM, preferably less about 100 nM and more preferably less than about 10 nM as determined by an in vitro assay such as a competition assay utilizing ErbB substrate analogous to those described by Jones et al., (1999) FEBS Letters 447:227-231. However, peptide ligands having an affinity for

25   ErbB2 of less than about 1 nM and preferably between about 1 pM and 1 nM are equally likely to be peptide ligands within the context of the present invention. In general a peptide ligand that binds ErbB2 as described above can be isolated and identified by any of a number of art standard techniques as described herein as for example by binding an immobilized ErbB2 molecule.

30   As mentioned, peptides ligands are amino acid sequences as described above which may contain naturally as well as non-naturally occurring amino acid residues. Therefore, so-called "peptide mimetics" and "peptide analogs" which may include non-amino acid chemical structures that mimic the structure of a particular amino acid or peptide may be peptide ligands within the context of

35   the invention. Such mimetics or analogs are characterized generally as exhibiting similar physical characteristics such as size, charge or hydrophobicity present in the appropriate spacial orientation as found in their peptide counterparts. A specific example of peptide mimetic compound is a compound in which the amide bond between one or more of the amino acids is

40   replaced by, for example, a carbon-carbon bond or other bond as is well known in the art (see, for example Sawyer, in Peptide Based Drug Design pp. 378-422 (ACS, Washington DC 1995).

    Therefore, the term "amino acid" within the scope of the present invention is used in its broadest sense and is meant to include naturally

occurring L α-amino acids or residues. The commonly used one and three letter abbreviations for naturally occurring amino acids are used herein (Lehninger, A.L., Biochemistry, 2d ed., pp. 71-92, (1975), Worth Publishers, New York). The term includes D-amino acids as well as chemically modified amino acids such

5   as amino acid analogs, naturally occurring amino acids that are not usually incorporated into proteins such as norleucine, and chemically synthesized compounds having properties known in the art to be characteristic of an amino acid. For example, analogs or mimetics of phenylalanine or proline, which allow the same conformational restriction of the peptide compounds as natural

10   Phe or Pro are included within the definition of amino acid. Such analogs and mimetics are referred to herein as "functional equivalents" of an amino acid. Other examples of amino acids are listed by Roberts and Vellaccio (The Peptides: Analysis, Synthesis, Biology,) Eds. Gross and Meiehofer, Vol. 5 p 341, Academic Press, Inc, N.Y. 1983, which is incorporated herein by reference.

15   Peptide ligands synthesized by, for example, standard solid phase synthesis techniques, are not limited to amino acids encoded by genes. Commonly encountered amino acids which are not encoded by the genetic code, include, for example, those described in International Publication No. WO 90/01940 such as, for example, 2-amino adipic acid (Aad) for Glu and Asp; 2-

20   aminopimelic acid (Apm) for Glu and Asp; 2-aminobutyric (Abu) acid for Met, Leu, and other aliphatic amino acids; 2-aminoheptanoic acid (Ahe) for Met, Leu and other aliphatic amino acids; 2-aminoisobutyric acid (Aib) for Gly; cyclohexylalanine (Cha) for Val, and Leu and Ile; homoarginine (Har) for Arg and Lys; 2,3-diaminopropionic acid (Dpr) for Lys, Arg and His; N-ethylglycine

25   (EtGly) for Gly, Pro, and Ala; N-ethylglycine (EtGly) for Gly, Pro, and Ala; N-ethylasparigine (EtAsn) for Asn, and Gln; Hydroxyllysine (Hyl) for Lys; allohydroxyllysine (AHyl) for Lys; 3-(and 4)hydoxyproline (3Hyp, 4Hyp) for Pro, Ser, and Thr; allo-isoleucine (AIle) for Ile, Leu, and Val; ρ-amidinophenylalanine for Ala; N-methylglycine (MeGly, sarcosine) for Gly, Pro,

30   and Ala; N-methylisoleucine (MeIle) for Ile; Norvaline (Nva) for Met and other aliphatic amino acids; Norleucine (Nle) for Met and other aliphatic amino acids; Ornithine (Orn) for Lys, Arg and His; Citrulline (Cit) and methionine sulfoxide (MSO) for Thr, Asn and Gln; N-methylphenylalanine (MePhe), trimethylphenylalanine, halo (F, Cl, Br, and I)phenylalanine,

35   triflourylphenylalanine, for Phe.

           Conservative amino acid subsitutions within the context of the invention are shown in Table 1 under the heading of "exemplary substitutions" and "preferred substitutions". If preferred substitutions do not result in a decrease or change in HER2 binding, then more substantial changes, denominated

40   "exemplary substitutions" in Table 1, or as further described herein, may be introduced and the products tested for HER2 binding.

Table 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	val; leu; ile	val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; asp, lys; arg	gln
Asp (D)	glu; asn	glu
Cys (C)	ser; ala	ser
Gln (Q)	asn; glu	asn
Glu (E)	asp; gln	asp
Gly (G)	ala	ala
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe; norleucine	leu
Leu (L)	norleucine; ile; val; met; ala; phe	ile
Lys (K)	arg; gln; asn	arg
Met (M)	leu; phe; ile	leu
Phe (F)	leu; val; ile; ala; tyr	tyr
Pro (P)	ala	ala
Ser (S)	thr	thr
Thr (T)	ser	ser
Trp (W)	tyr; phe	tyr
Tyr (Y)	trp; phe; thr; ser	phe
Val (V)	ile; leu; met; phe; ala; norleucine	leu

Peptide ligands within the context of the present invention are preferably non-naturally occurring amino acid sequences. By non-naturally occurring is meant that the amino acid sequence of the particular peptide ligand is not found in nature. According to the present invention the term "non-naturally occurring" is used to refer to a peptide ligand that corresponds to a non-native or non-naturally occurring amino acid sequence. Peptide ligands of this variety may be produced or selected using a variety of techniques well known to the skilled artisan. For example, constrained or

unconstrained peptide libraries may be randomly generated and displayed on phage utilizing art standard techniques (for example, Lowman et al., (1998) Biochemistry 37:8870-8878).

At least three distinct species of peptide ligands can be distinguished based upon function associated with binding ErbB2. They will be referred to herein as "neutral," "agonist" and "antagonist" peptide ligands. In general, a neutral peptide ligand functions to bind ErbB2 as described above. Neutral peptide ligands are preferred in aspects of the present invention where targeting of a particular cell type bearing ErbB2 with, for example, a cytotoxic agent or an enzyme is desired.

In general, an "agonist" peptide ligand in addition to binding ErbB2, has a direct effect on an ErbB2 bearing cell. Preferably the agonist peptide ligand will bind ErbB2 as described and, as well, initiate or mediate an event associated with the ErbB2-ErbB3 protein complex and/or the ErbB2-ErbB4 protein complex such as, for example, the ability to cause the intracellular kinase domain to phosphorylate tyrosine residues in the ErbB2 receptor complex. Additionally, binding of the agonist peptide ligand maybe associated with dimerization of the ErbB2 receptor. The ability to induce ErbB2 receptor mediated phosphorylation or ErbB2 receptor dimerization can be quantified using art standard techniques such as tyrosine phosphorylation assays and SDS PAGE.

By contrast, an "antagonist" peptide ligand acts to reduce the activity associated with a natural ligand binding the ErbB2-ErbB3 protein complex and/or ErbB2-ErbB4 protein complex, for example, the cellular response induced by the binding of the native ligand, by for example, binding to and blocking the association of ErbB2-ErbB3 protein complex and/or ErbB2-ErbB4 protein complex with a native or naturally occurring ligand.

However, the foregoing classes are not limiting. For example, in certain embodiments, binding of a peptide ligand causes internalization of the ErbB2 receptor, or the induction of associated cellular events such as the induction of programmed cell death or apoptosis. Peptide ligands that initiate internalization of ErbB2 or an ErbB2 receptor complex are especially useful in embodiments of the invention requiring intracellular delivery of a cytotoxic agent as described herein below.

The phrase "induces apoptosis" or "capable of inducing apoptosis" refers to the ability of a compound to induce programmed cell death as determined by binding of annexin V, fragmentation of DNA, cell shrinkage, dilation of endoplasmic reticulum, cell fragmentation, and/or formation of membrane vesicles (called apoptotic bodies). The cell is one which expresses or overexpresses the ErbB2 receptor. Preferably the "cell" is a tumor cell, e.g. a breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic or bladder cell. In vitro, the cell may be a SKBR3, BT474, Calu 3 cell, MDA-MB-453, MDA-MB-361 or SKOV3 cell. Various methods are available for evaluating the cellular events associated with apoptosis. For example, phosphatidyl serine (PS) translocation can be measured by annexin

binding; DNA fragmentation can be evaluated through DNA laddering; and nuclear/chromatin condensation along with DNA fragmentation can be evaluated by any increase in hypodiploid cells. Preferably, the compound which induces apoptosis is one which results in about 2 to 50 fold, preferably about 5 to 50 fold, and most preferably about 10 to 50 fold, induction of annexin binding relative to untreated cell in an annexin binding assay using BT474 cells.

"Heregulin" (HRG) when used herein refers to a polypeptide which activates the ErbB2-ErbB3 and ErbB2-ErbB4 protein complexes (i.e. induces phosphorylation of tyrosine residues in the complex upon binding thereto). Various polypeptides encompassed by this term are disclosed in Jones et al., (1999) FEBS Letters 447:227-231; Holmes et al., (1992) Science, 256:1205-1210; WO 92/20798; Wen et al., (1994) Mol. Cell. Biol., 14(3):1909-1919; and Marchionni et al., (1993) Nature, 362:312-318, for example. The term includes biologically active fragments and/or variants of a naturally occurring HRG polypeptide, such as an EGF-like domain fragment thereof (e.g. $HRG\beta1_{177-244}$).

The "ErbB2-ErbB3 protein complex" and "ErbB2-ErbB4 protein complex" are noncovalently associated oligomers of the ErbB2 receptor and the ErbB3 receptor or ErbB4 receptor, respectively. The complexes form on a cell expressing both of these receptors and can be isolated by immunoprecipitation and analyzed by SDS-PAGE as described in Sliwkowski et al., (1994) J. Biol. Chem., 269(20):14661-14665.

The term "multimerization domain" as used in particular aspects of the present invention, is meant to refer to the portion of the molecule to which the peptide ligand is joined, either directly or through a "linker domain." The multimerization domain is an amino acid domain which, according to preferred embodiments, facilitates the interaction of two or more multimerization domains. While the multimerization domain promotes the interaction between two or more multimerization domains, there is no requirement within the context of the present invention that the peptide ligand joined to a multimerization domain be present as a portion of a multimer.

According to preferred aspects of the present invention the multimerization domain is a polypeptide which promotes the stable interaction of two or more multimerization domains. By way of example and not limitation, a multimerization domain may be an immunoglobulin sequence, such as an immunoglobulin constant region, a leucine zipper, a hydrophobic region, a hydrophilic region, a polypeptide comprising a free thiol which forms an intermolecular disulfide bond between two or more multimerization domains or, for example a "protuberance-into-cavity" domain described in U.S. Patent 5,731,168. In that patent, protuberances are constructed by replacing small amino acid side chains from the interface of a first polypeptide with a larger side chain (for example a tyrosine or tryptophan). Compensatory cavities of identical or similar size to the protuberances are optionally created on the interface of a second polypeptide by replacing large amino acid side chains with smaller ones (for example alanine or threonine).

Therefore, in a preferred aspect, the multimerization domain provides
that portion of the molecule which promotes or allows stable interaction of two
or more multimerization domains and promotes or allows the formation of dimers
and other multimers from monomeric multimerization domains.   Preferably,
according to this aspect of the invention, multimerization domains are
immunoglobulin constant region domains.   Immunoglobulin constant domains
provide the advantage of improving in vivo circulating half-life of the
compounds of the invention and optionally allow the skilled artisan to
incorporate an "effector function" as described herein below into certain
aspects of the invention.

Throughout the present specification and claims, the numbering of the
residues in an immunoglobulin heavy chain is that of the EU index as in Kabat
et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health
Service, National Institutes of Health, Bethesda, MD (1991), expressly
incorporated herein by reference.   The "EU index as in Kabat" refers to the
residue numbering of the human IgG1 EU antibody.

"Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having
the same structural characteristics.   While antibodies exhibit binding
specificity to a specific antigen, immunoglobulins include both antibodies and
other antibody-like molecules which lack antigen specificity.   Polypeptides of
the latter kind are, for example, produced at low levels by the lymph system
and at increased levels by myelomas.

"Antibodies"   and   "immunoglobulins"   are   usually   heterotetrameric
glycoproteins of about 150,000 Daltons, composed of two identical light (L)
chains and two identical heavy (H) chains.   Each light chain is linked to a
heavy chain by one covalent disulfide bond, while the   number of disulfide
linkages varies between the heavy chains of different immunoglobulin isotypes.
Each heavy and light chain also has regularly spaced intrachain disulfide
bridges.   Each heavy chain has an amino (N) terminal variable domain (VH)
followed by carboxy (C) terminal constant domains.   Each light chain has a
variable N-terminal domain (VL) and a C-terminal constant domain; the constant
domain of the light chain (CL) is aligned with the first constant domain (CH1)
of the heavy chain, and the light chain variable domain is aligned with the
variable domain of the heavy chain.   According to the domain definition of
immunoglobulin polypeptide chains, light (L) chains have two conformationally
similar domains VL and CL; and heavy chains have four domains (VH, CH1, CH2,
and CH3) each of which has one intrachain disulfide bridge.

Depending on the amino acid sequence of the constant (C) domain of the
heavy chains, immunoglobulins can be assigned to different classes.   There are
five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM.   The
immunoglobulin class can be further divided into subclasses (isotypes), e.g.,
IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2.   The heavy-chain constant domains that
correspond to the different classes of immunoglobulins are α, δ, ϵ, γ, and μ
domains respectively.   The light chains of antibodies from any vertebrate

species can be assigned to one of two distinct types called kappa (κ) or lambda (λ), based upon the amino acid sequence of their constant domains. Sequence studies have shown that the μ chain of IgM contains five domains VH, CHμ1, CHμ2, CHμ3, and CHμ4. The heavy chain of IgE (ε) also contains five domains.

The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known. Of these IgA and IgM are polymeric and each subunit contains two light and two heavy chains. The heavy chain of IgG (γ) contains a length of polypeptide chain lying between the CHγ1 and CHγ2 domains known as the hinge region. The α chain of IgA has a hinge region containing an O-linked glycosylation site and the μ and ε chains do not have a sequence analogous to the hinge region of the γ and α chains, however, they contain a fourth constant domain lacking in the others. The domain composition of immunoglobulin chains can be summarized as follows:

Light Chain   λ = Vλ Cλ

κ = Vκ Cκ

Heavy Chain IgG (γ) = VH CHγ1, hinge CHγ2 CHγ3

IgM (μ) = VH CHμ1 CHμ2 CHμ3 CHμ4

IgA (α) = VH CHα1 hinge CHα2 CHα3

IgE (ε) = VH CHε1 CHε2 CHε3 CHε4

IgD (δ) = VH CHδ1 hinge CHδ2 CHδ3

The "CH2 domain" of a human IgG Fc region (also referred to as "Cγ2" domain) usually extends from about amino acid 231 to about amino acid 340. The CH2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native IgG molecule.

The "CH3 domain" comprises the stretch of residues C-terminal to a CH2 domain in an Fc region (i.e. from about amino acid residue 341 to about amino acid residue 447 of an IgG).

"Hinge region" is generally defined as stretching from Glu216 to Pro230 of human IgG1 (Burton, *Molec. Immunol.*22:161-206 (1985)). Hinge regions of other IgG isotypes may be aligned with the IgG1 sequence by placing the first and last cysteine residues forming inter-heavy chain S-S bonds in the same positions.

The "lower hinge region" of an Fc region is normally defined as the stretch of residues immediately C-terminal to the hinge region, i.e. residues 233 to 239 of the Fc region.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments or regions, each with a single antigen-binding site, and a residual "Fc" fragment or region (Figure 1). Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The Fc region of an immunoglobulin generally comprises two constant domains, CH2 and CH3, as shown, for example, in Fig. 1. A "native Fc region

sequence" comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. Native human Fc region sequences are shown in Fig. 2 & 3 and include but are not limited to the human IgG1 Fc region (non-A and A allotypes); human IgG2 Fc region; human IgG3 Fc region; and human IgG4 Fc region as well as naturally occurring variants thereof. Native murine Fc regions sequences are shown in Fig. 2A.

Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen. The Fab fragment contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

A "functional Fc region" possesses an "effector function" of a native Fc region. Exemplary "effector functions" include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor; BCR), etc. Such effector functions generally require the Fc region to be combined with a binding domain (e.g. a peptide ligand) and can be assessed using various assays known in the art.

By introducing the appropriate amino acid sequence modifications in a parent or native Fc region, one can generate a variant Fc region which (a) mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of human effector cells more effectively and/or (b) binds an Fc gamma receptor (FcγR) with better affinity than the parent polypeptide. Such Fc region variants will generally comprise at least one amino acid modification in the Fc region. The variant Fc region may include two, three, four, five, etc substitutions therein.

Several antibody effector functions are mediated by Fc receptors (FcRs), which bind the Fc region of an antibody. FcRs are defined by their specificity for immunoglobulin isotypes; Fc receptors for IgG antibodies are referred to as FcγR, for IgE as FcεR, for IgA as FcαR and so on. Three subclasses of FcγR have been identified: FcγR I (CD64), FcγR II (CD32) and FcγR III (CD16). Because each FcγR subclass is encoded by two or three genes, and alternative RNA spicing leads to multiple transcripts, a broad diversity in FcγR isoforms exists. These different FcR subtypes are expressed on different cell types (reviewed in Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991)). For example, in humans, FcγRIIIB is found only on neutrophils, whereas FcγRIIIA is found on macrophages, monocytes, natural killer (NK) cells, and a subpopulation

of T-cells. Notably, FcγRIIIA is the only FcR present on NK cells, one of the cell types implicated in ADCC.

The binding site on human and murine antibodies for FcγR have been previously mapped to the lower hinge region (residues 233-239: EU index numbering as in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). Woof et al. Molec. Immunol. 23:319-330 (1986); Duncan et al. Nature 332:563 (1988); Canfield and Morrison, J. Exp. Med. 173:1483-1491 (1991); Chappel et al., Proc. Natl. Acad. Sci USA 88:9036-9040 (1991).

"C1q" is a polypeptide that includes a binding site for the Fc region of an immunoglobulin. C1q together with two serine proteases, C1r and C1s, forms the complex C1, the first component of the complement dependent cytotoxicity (CDC) pathway.

"Antibody-dependent cell-mediated cytotoxicity" and "ADCC" refer to a cell-mediated reaction in which nonspecific cytotoxic cells that express FcRs (e.g. Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cell. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells in summarized is Table 3 on page 464 of Ravetch and Kinet, Annu. Rev. Immunol 9:457-92 (1991).

As used herein, the term "salvage receptor binding ligand" refers to an ligand of the Fc region of an IgG molecule (e.g., IgG_1, IgG_2, IgG_3, or IgG_4) that is responsible for increasing the in vivo serum half-life of the IgG molecule (U.S. Patent No. 5,739,277, issued April 14, 1998).

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc. Preferably, the mammal is human.

A "disorder" is any condition that would benefit from treatment with the compositions comprising the peptide ligands of the invention. This includes chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include benign and malignant tumors; leukemias and lymphoid malignancies; neuronal, glial, astrocytal, hypothalamic and other glandular, macrophagal, epithelial, stromal and blastocoelic disorders; and inflammatory, angiogenic and immunologic disorders.

The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma,

lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer,

5   colon cancer, colorectal cancer, endometrial carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer.

As used herein, the term "parenteral" refers to introduction of a compound of the invention into the body by other than the intestines, and in

10   particular, intravenous (i.v.), intraarterial (i.a.), intraperitoneal (i.p.), intramuscular (i.m.), intraventricular, and subcutaneous (s.c.) routes.

Modes for Carrying out the Invention

Peptide Ligands

Peptide ligands within the context of the present invention bind ErbB2

15   and can be identified by their ability to compete for binding ErbB2 in an _in vitro_ assay with a peptide ligand having the general formula:

$$Xaa_{(1-14)}-Cys-Xaa_{16}-Gly-Pro-Gly-Cys-Xaa_{(21-27)} \quad (SEQ\ ID\ NO:90)$$
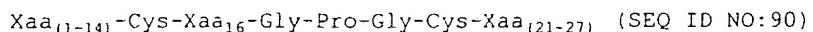
wherein $Xaa_{(1-14)}$ is absent or between one and fourteen amino acids; Xaa_{16} is selected from the group consisting of Met, Thr, Cys and Ile and is preferably

20   Ile; $Xaa_{(21-27)}$ is absent or between one and 7 amino acids. Preferably the peptide ligand will compete for binding ErbB2 as described with a peptide ligand having the following general formula:

$$Xaa_{(1-3)}-Glu-Xaa_5-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Xaa_{14}-Xaa_{15}-Leu-Xaa_{(17-20)}$$
(SEQ ID NO:91)
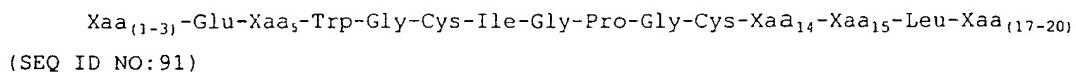
25   wherein $X_{(1-3)}$ and $X_{(17-20)}$ are, independently, absent or between one and three and one and four amino acids respectively and Xaa_1, Xaa_2 and Xaa_3 are amino acids. In particular embodiments, the peptide ligand of the present invention will compete with any of the peptide ligands represented in SEQ ID NO: 1 – SEQ ID NO: 44 and SEQ ID NO: 57 – SEQ ID NO: 69 decribed herein and preferably will

30   compete with SEQ ID NO: 14 for binding ErbB2.

As will be appreciated from the foregoing, the term "compete" and "ability to compete" are relative terms. Thus the terms, when used to describe the peptide ligands of the present invention, refer to peptide ligands that produce a 50% inhibition of binding of, for example, SEQ ID NO:14, when present

35   at 50 μM, preferably when present at 1 μM, more preferably 100 μM, and preferably when present at 1nM or less in a standard competition assay as described herein. Such peptide ligands will generally bind ErbB2 with an affinity of less than about 1 μM, preferably less about 100 nM and more preferably less than about 10 nM as determined by a standard competition assay

40   such as the one described in the Example sections. However, peptide ligands having an affinity for ErbB2 of less than about 1 nM and preferably between about 1 pM and 1 nM are equally likely to be peptide ligands within the context of the present invention.

For _in vitro_ assay systems to determine whether a compound has the ability to compete or competes with a peptide ligand described above, the skilled artisan can employ any of a number of standard competition assays. Such procedures include but are not limited to competitive assay systems using
5   techniques such as radioimmunoassays, enzyme immunoassays (EIA), preferably the enzyme linked immunosorbent assay (ELISA), "sandwich" immunoassays, immunoradiometric assays, fluorescent immunoassays, and immunoelectrophoresis assays, to name but a few.

By way of exemplification and not limitation an ELISA assay can be
10   conducted wherein microtiter plates (for example, Nunc Maxisorp™ 96-well plates, Inter Med, Denmark) are coated with HER2-ECD in 50 mM ammonium bicarbonate, pH 9.3, using 5 µg/ml, overnight at 4°C. Wells can be blocked using PBS containing 1% BSA (PBS-BSA) for 1 hr at 25°C. Candidate peptide ligands are titered in PBS-BSA and tested for their ability to block the
15   binding of peptide 1.1.FI (SEQ ID NO: 14) produced as a Z protein fusion (1.1.FI-Z) as described herein and biotinylated (1.1.FI-Zb) to immobilized HER2-ECD. Following an incubation of about 1 hour the plate is washed with PBS-TWEEN and Strepavidin-HRP is added for about 30 min. Plates are washed again with PBS-TWEEN and the bound HRP assayed using ABTS/H_2O_2 substrate. The
20   change in absorbance at 405 nm is monitored. Decrease in absorbance is plotted vs. sample concentration and an IC_{50} for each candidate peptide ligand is determined.

As previously noted, preferred peptide ligands of the present invention are non-naturally occuring peptide ligands of between about 10 and 30 amino
25   acid residues and preferably about 20 amino acid residues. Especially preferred peptide ligands as described above consist of naturally occurring amino acids and can be generated using standard recombinant and synthetic techniques well known to the skilled artisan.

Preferred peptide ligands have the general formula:
30   Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Cys-Xaa-Xaa-Xaa-Xaa-Cys-Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Xaa. Somewhat more particularly, preferred peptide ligands have the formula:

$Xaa_{(1-7)}$-Cys-Xaa_9-Gly-Pro-Gly-Cys-$Xaa_{(14-20)}$ (SEQ ID NO:92)

wherein $X_{(1-7)}$ and $Xaa_{(14-20)}$ are, independently, absent or between one and seven amino acids and Xaa_9 is an amino acid selected from the group consisting of
35   Met, Ile and Thr and is preferably Ile. Somewhat more particularily, preferred peptide ligands have the formula:

$Xaa_{(1-5)}$-Trp-Xaa_7-Cys-Xaa_9-Gly-Pro-Gly-Cys-$Xaa_{(14-20)}$ (SEQ ID NO:93)

wherein $Xaa_{(1-5)}$ is absent or between one and five amino acids and Xaa_7 is selected from the group consisting of Asp, Gly, Glu and His and preferably Gly.
40   More preferred according to this aspect of the invention, are peptide ligands having the following formula are preferred:

$Xaa_{(1-3)}$-Glu-Xaa_5-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Xaa_{14}-Xaa_{15}-Leu-$Xaa_{(17-20)}$ (SEQ ID NO:91)

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wherein $Xaa_{(1-3)}$- is absent or between one and three amino acids, Xaa_5 is an amino acid, Xaa_{13} is an amino acid, Xaa_{14} is an amino acid and -$Xaa_{(17-20)}$ is absent or between one and four amino acids. Exemplary peptide ligands according to this aspect of the invention are peptide ligands wherein $X_{(1-3)}$-
is absent or selected from the group consisting of:
Gln-Arg-Asn-, Leu-Ser-Pro-, Glu-Asn-Trp-, Ala-Ser-His-, Lys-Leu-Asn-, Thr-Gln-Ala-, Ala-Pro-Arg-, Gln-Val-Tyr-, Arg-Thr-Glu-, Phe-Ala-Gly-, Thr-Ala-Arg-, Arg-Pro-His-, Asn-Val-Cys-, Cys-Ile-Asp-, Tyr-Glu-Trp-, Arg-Trp-Asp-, His-Trp-Met-, Asn-Trp-Pro-, Phe-Asn-Trp-, Phe-Ser-Gly-, Gly-Gly-Trp-, Leu-Trp-Phe-, Gly-Ile-Pro-, Trp-Trp-Thr-, Leu-Gly-Trp-, Ser-Pro-Trp-, Arg-Gly-Trp-, Tyr-Glu-Phe-, Tyr-Glu-Gly-, Tyr-Glu-Val-, Tyr-Ser-Phe-, Tyr-Asp-Phe-, Asp-Glu-Val-, Ser-Glu-Val-, Phe-Glu-Phe-, and His-Asp-Val-; Xaa_5 is selected from the group consisting of Ala, Thr, Met, Val, Arg, Glu, Asp, Ser, Gln, Pro, Gly, Phe and Lys; Xaa_{14} is selected from the group consisting of Glu, Lys, Arg, Asp, Ser, Ala, Asn, Thr, Gly, Pro, Val and Gln, Xaa_{15} is selected from the group consisting of Met, Phe, Ala, Cys, Gln, Glu, Trp, Leu, Val, Tyr, Thr, Ser and Asn, and -$Xaa_{(17-20)}$ is a four amino acid peptide having the following exemplary sequences:
-Phe-Gly-Ala-His (SEQ ID NO:94)
-Phe-Asp-Ala-His (SEQ ID NO:95)
-Leu-Glu-Ala-His (SEQ ID NO:96)
-Phe-Glu-Gly-His (SEQ ID NO:97)
-Phe-Gly-Ala-Leu (SEQ ID NO:98)
-Phe-Glu-Ala-Tyr (SEQ ID NO:99)
-Phe-Ala-Gly-His (SEQ ID NO:100) and
-Phe-Glu-Ala-Phe (SEQ ID NO:101).
Preferably -$Xaa_{(17-20)}$ is a four amino acid peptide having the following formula:
$$-Xaa_{17}-Xaa_{18}-Cys-Xaa_{20}.$$
Exemplary peptide ligands according to this aspect of the invention are peptide ligands wherein -$Xaa_{(17-20)}$ is selected from the group consisting of:
-Gln-Ala-Cys-Met (SEQ ID NO:102)
-Leu-Gln-Cys-Trp (SEQ ID NO:103)
-Met-Ser-Cys-Val (SEQ ID NO:104)
-Leu-Arg-Cys-Ile (SEQ ID NO:105)
-Gln-Ala-Cys-Leu (SEQ ID NO:106)
-Leu-Ser-Cys-Leu (SEQ ID NO:107)
-Ile-Gly-Cys-Leu (SEQ ID NO:108)
-Leu-Ala-Cys-Leu (SEQ ID NO:109)
-Leu-Ser-Cys-Ile (SEQ ID NO:110)
-Met-Asn-Cys-Leu (SEQ ID NO:111)
-Leu-Arg-Cys-Leu (SEQ ID NO:112)
-Leu-Lys-Cys-Leu (SEQ ID NO:113)
-Leu-Gly-Cys-Leu (SEQ ID NO:114)

-Leu-Asn-Cys-Ile (SEQ ID NO:115)

-Met-Gly-Cys-Leu (SEQ ID NO:116) and

-Met-Ala-Cys-Leu (SEQ ID NO:117).

     According to another embodiment of this aspect of the invention are peptide ligands wherein $-Xaa_{(17-20)}$ is a four amino acid peptide having the following formula:

     $-Cys-Xaa_{18}-Xaa_{19}-Cys$

wherein Xaa_{18} is an amino acid and Xaa_{19} is an amino acid. According to this aspect of the invention, exemplary peptide ligands comprise an $-Xaa_{(17-20)}$ selected from the group consisting of:

-Cys-Ala-Trp-Cys (SEQ ID NO:118)

-Cys-Ser-Trp-Cys (SEQ ID NO:119)

-Cys-Glu-Pro-Cys (SEQ ID NO:120)

-Cys-Asp-Trp-Cys (SEQ ID NO:121)

-Cys-Glu-Trp-Cys (SEQ ID NO:122)

-Cys-Asn-Trp-Cys (SEQ ID NO:123) and

-Cys-Gly-Trp-Cys (SEQ ID NO:124).

     Exemplary peptide ligands according to this aspect of the invention include:

Gln-Arg-Asn-Glu-Ala-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Glu-Met-Leu-Cys-Ala-Trp-Cys (SEQ ID NO:1)

Leu-Ser-Pro-Glu-Thr-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Glu-Met-Leu-Cys-Ser-Trp-Cys (SEQ ID NO:2)

Glu-Asn-Trp-Glu-Met-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Lys-Phe-Leu Cys-Glu-Pro-Cys (SEQ ID NO:3)

Glu-Val-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Lys-Ala-Leu-Cys-Asp-Trp-Cys (SEQ ID NO:4)

Glu-Arg-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Arg-Met-Leu-Cys-Glu-Trp-Cys (SEQ ID NO:5)

Glu-Val-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Asp-Met-Leu-Cys-Asn-Trp-Cys (SEQ ID NO:6)

Glu-Val-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Ser-Met-Leu-Cys-Gly-Trp-Cys (SEQ ID NO:7)

Xaa-Xaa-Xaa-Glu-Xaa-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Xaa-Met-Leu-Cys-Xaa-Trp-Cys (SEQ ID NO:8)

Ala-Ser-His-Glu-Val-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Lys-Cys-Leu-Gln-Ala-Cys-Met (SEQ ID NO:9)

Lys-Leu-Asn-Glu-Glu-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Ala-Cys-Leu Leu-Gln-Cys-Trp (SEQ ID NO:10)

Lys-Leu-Asn-Glu-Asp-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Ala-Cys-Leu-Leu-Xaa-Cys-Xaa (SEQ ID NO:11)

Thr-Gln-Ala-Glu-Arg-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Glu-Cys-Leu-Met-Ser-Cys-Val (SEQ ID NO:12)

Ala-Pro-Arg-Glu-Val-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Ala-Cys-Leu-Leu-Arg-Cys-Ile
(SEQ ID NO:13)

Gln-Val-Tyr-Glu-Ser-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Ala-Cys-Leu-Gln-Ala-Cys-Leu
(SEQ ID NO:14)

5   Arg-Thr-Glu-Glu-Gln-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Arg-Cys-Leu Leu-Ser-Cys-Leu
(SEQ ID NO:15)

Phe-Ala-Gly-Glu-Ser-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Glu-Cys-Leu-Ile-Gly-Cys-Leu
(SEQ ID NO:16)

Thr-Ala-Arg-Glu-Val-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Asn-Cys-Leu-Leu-Ala-Cys-Leu

10   (SEQ ID NO:17)

Arg-Pro-His-Glu-Pro-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Ser-Cys-Leu- Leu-Ser-Cys-
Ile (SEQ ID NO:18)

Glu-Val-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Glu-Cys-Leu-Met-Asn-Cys-Leu (SEQ ID
NO:19)

15   Glu-Gly-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Glu-Cys-Leu-Leu-Arg-Cys-Leu (SEQ ID
NO:20)

Glu-Gly-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Gly-Cys-Leu-Leu-Lys-Cys-Leu (SEQ ID
NO:21)

Glu-Pro-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Ala-Cys-Leu-Leu-Gly-Cys-Leu (SEQ ID

20   NO:22)

Glu-Glu-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Ala-Cys-Leu-Leu-Asn-Cys-Ile (SEQ ID
NO:23)

Glu-Gln-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Lys-Cys-Leu-Met-Gly-Cys-Leu (SEQ ID
NO:24)

25   Glu-Gln-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Gly-Cys-Leu-Leu-Arg-Cys-Leu (SEQ ID
NO:25)

Glu-Ala-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Gly-Cys-Leu-Met-Ala-Cys-
Leu (SEQ ID NO:26)

Xaa-Xaa-Xaa-Glu-Xaa-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Xaa-Cys-Leu-Leu-Xaa-Cys-Leu

30   (SEQ ID NO:27)

Asn-Val-Cys-Glu-Phe-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Ala-Gln-Leu-Cys (SEQ ID
NO:28)

Cys-Ile-Asp-Glu-Thr-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Glu-Glu-Leu Arg-Cys-Lys-Arg
(SEQ ID NO:29)

35   Tyr-Glu-Trp-Glu-Gly-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Pro-Ala-Leu-Gly-Phe-Gly-Tyr
(SEQ ID NO:30)

Arg-Trp-Asp-Glu-Glu-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Glu-Trp-Leu-Val-Val-Arg-Lys
(SEQ ID NO:31)

His-Trp-Met-Glu-Arg-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Gly-Phe-Leu(SEQ ID NO:32)

40   Asn-Trp-Pro-Glu-Gly-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Lys-Leu-Leu(SEQ ID NO:33)

Phe-Asn-Trp-Glu-Lys-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Arg-Thr-Leu(SEQ ID NO:34)

Phe-Ser-Gly-Glu-Arg-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Gln-Val-Leu(SEQ ID NO:35)

Gly-Gly-Trp-Glu-Gly-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Arg-Tyr-Leu(SEQ ID NO:36)

Leu-Trp-Phe-Glu-Arg-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Thr-Xaa-Leu(SEQ ID NO:37)

Gly-Ile-Pro-Glu-Gly-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Glu-Trp-Leu(SEQ ID NO:38)

Trp-Trp-Thr-Glu-Arg-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Ser-Met-Leu(SEQ ID NO:39)

Xaa-Cys-Xaa-Glu-Arg-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Ser-Met-Leu(SEQ ID NO:40)

Leu-Gly-Trp-Glu-Arg-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Arg-Ala-Leu(SEQ ID NO:41)

5   Ser-Pro-Trp-Glu-Gly-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Arg-Trp-Leu(SEQ ID NO:42)

Arg-Gly-Trp-Glu-Gly-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Ser-Phe-Leu(SEQ ID NO:43)

Xaa-Xaa-Trp-Glu-Xaa-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Xaa-Xaa-Leu- Xaa-Xaa-Xaa-
Xaa (SEQ ID NO:44)

Tyr-Glu-Val-Glu-Ala-Trp-Asp-Cys-Met-Gly-Pro-Gly-Cys-Ala-Asn-Leu-Phe-Glu-Ala-His

10   (SEQ ID NO:57)

Tyr-Glu-Phe-Glu-Gly-Trp-Asp-Cys-Met-Gly-Pro-Gly-Cys-Ala-Ser-Val-Phe-Gly-Ala-His
(SEQ ID NO:58)

Tyr-Glu-Gly-Glu-Ser-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Ala-Ser-Leu-Phe-Asp-Ala-His
(SEQ ID NO:59)

15   Tyr-Glu-Val-Glu-Val-Trp-Glu-Cys-Ile-Gly-Pro-Gly-Cys-Gly-Tyr-Leu-Phe-Gly-Ala-His
(SEQ ID NO:60)

Tyr-Glu-Val-Glu-Gly-Trp-Gly-Cys-Met-Gly-Pro-Gly-Cys-Ala-Phe-Leu-Leu-Glu-Ala-His
(SEQ ID NO:61)

Tyr-Ser-Phe-Glu-Gly-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Ala-Tyr-Leu-Phe-Glu-Gly-His

20   (SEQ ID NO:62)

Tyr-Asp-Phe-Glu-Gly-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Gly-Asn-Leu-Leu-Glu-Ala-His
(SEQ ID NO:63)

Tyr-Asp-Phe-Glu-Gly-Trp-Asp-Cys-Thr-Gly-Pro-Gly-Cys-Ala-Tyr-Leu-Phe-Glu-Gly-His
(SEQ ID NO:64)

25   Asp-Glu-Val-Glu-Ser-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Ala-Tyr-Leu-Phe-Gly-Ala-Leu
(SEQ ID NO:65)

Ser-Glu-Val-Glu-Val-Trp-His-Cys-Ile-Gly-Pro-Gly-Cys-Val-Tyr-Leu-Phe-Glu-Ala-Tyr
(SEQ ID NO:66)

Phe-Glu-Phe-Glu-Gly-Trp-Glu-Cys-Met-Gly-Pro-Gly-Cys-Ala-Glu-Leu-Phe-Ala-Gly-His

30   (SEQ ID NO:67)

His-Asp-Val-Glu-Gly-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Ala-Asp-Leu-Phe-Glu-Ala-Phe
(SEQ ID NO:68)

Tyr-Glu-Phe-Glu-Gly-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Ala-Tyr-Leu-Phe-Glu-Ala-His
(SEQ ID NO:69)

35   including conservative amino acid substitutions within the foregoing exemplary
amino acid sequences.

Further preferred peptide compounds also have the general formula:
Cys-Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Cys-Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Cys-Cys-Xaa-Xaa-Xaa-
Cys-Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Cys (SEQ ID NO:125). Somewhat more particularly

40   the formula:
$Xaa_{(1-10)}$-Glu-Xaa_{12}-Trp-Xaa_{14}-Cys-Cys-Gly-Pro-Gly-Cys-Xaa_{21}-Xaa_{22}-Xaa $_{23}$-$Xaa_{(24-27)}$
(SEQ ID NO:126)
wherein $Xaa_{(1-10)}$ is absent or between 1 and 10 amino acids, Xaa_{12} is an amino
acid, Xaa_{14} is an amino acid, Xaa_{21} is an amino acid, Xaa_{22} is an amino acid,

Xaa$_{23}$ is an amino acid selected from the group consisting of Val and Leu and
Xaa$_{(24-27)}$ is absent or between one and four amino acids. Preferably, according
to this aspect of the invention, Xaa$_{12}$ is an amino acid selected from the group
consisting of Val, Leu, Ser, Trp; Xaa$_{14}$ is an amino acid selected from the
group consisting of Gln, Glu, Asp and His; Xaa$_{21}$ is an amino acid selected from
the group consisting of Gly and Glu; Xaa$_{22}$ is an amino acid selected from the
group consisting of Trp, Leu and Phe and Xaa$_{23}$ is Val.

Prefered peptide ligands according to this aspect of the invention
include those wherein Xaa$_{(1-10)}$ is 10 amino acids having the following formula:
Cys-Xaa$_{(2-7)}$-Cys-Xaa$_9$-Gly- and wherein Xaa$_{(24-27)}$ is four amino acids having the
following formula:
Xaa$_{(24-26)}$-Cys and wherein Xaa$_{(2-7)}$ is six amino acids and Xaa$_{(24-26)}$ is 3 amino
acids.

Preferred according to this aspect of the invention are peptide ligands
wherein Xaa$_{(1-10)}$ has the following formula:
Cys-Xaa$_2$-Trp-Val-Xaa$_5$-Xaa$_6$-Xaa$_7$-Cys-Xaa$_9$-Gly- (SEQ ID NO:127) wherein
Xaa$_2$ is an amino acid selected from the group consisting of Ala and Ser, Xaa$_5$
is an amino acid selected from the group consisting of Ser, Leu, Ala, Arg and
Val, Xaa$_6$ is an amino acid selected from the group consisting of Phe, Val and
Leu, Xaa$_7$ is an amino acid selected from the group consisting of Asp, Gln, Tyr,
Trp, Leu and His and Xaa$_9$ is an amino acid selected from the group consisting
of Gly, Phe and Leu.

Preferred among this group of peptides are peptide ligands wherein Xaa$_{(1-10)}$ has the following formula:
Cys-Ala-Trp-Val-Leu-Xaa$_6$-Xaa$_7$-Cys-Gly-Gly- (SEQ ID NO:128) and wherein Xaa$_{(24-26)}$
has the following formula:
Xaa$_{24}$-Xaa$_{25}$-Xaa$_{26}$ and Xaa$_{24}$ is an amino acid selected from the group consisting
of Trp, Val, Gly and Ala, preferably Val; Xaa$_{25}$ is an amino acid selected from
the group consisting of Asn, Lys, Asp, Glu and His, preferably Asn and Xaa$_{26}$
is an amino acid selected from the group consisting of Ala, Ser and Val and
preferably Ala.

By way of exemplification and not limitation the following are suitable
peptide ligands within the context of the present invention:
Cys-Ser-Trp-Val-Leu-Val-Gln-Cys-Gly-Gly-Glu-Trp-Trp-His-Cys-Cys- Gly-Leu-Gly-
Cys-Gly-Leu-Val-Val-Asn-Ala-Cys (SEQ ID NO: 45)
Cys-Ala-Trp-Val-Ser-Phe-Glu-Cys-Gly-Gly-Glu-Val-Trp-His-Cys-Cys-Gly-Leu-Gly-
Cys-Gly-Trp-Val-Trp-Lys-Ala-Cys (SEQ ID NO: 46)
Cys-Ala-Trp-Val-Leu-Val-Gln-Cys-Gly-Gly-Glu-Trp-Trp-His-Cys-Cys-Gly-Pro-Gly-
Cys-Glu-Phe-Val-Val-Asp-Ala-Cys (SEQ ID NO: 47)
Cys-Ala-Trp-Val-Ala-Val-Tyr-Cys-Gly-Gly-Glu-Leu-Trp-His-Cys-Cys-Gly-Pro-Gly-
Cys-Gly-Phe-Val-Val-Asp-Ser-Cys (SEQ ID NO: 48)
Cys-Ala-Trp-Val-Arg-Val-Trp-Cys-Phe-Gly-Glu-Trp-Trp-Asp-Cys-Cys-Gly-Leu-Gly-
Cys-Gly-Trp-Val-Val-Asn-Val-Cys (SEQ ID NO: 49)

Cys-Ala-Trp-Val-Arg-Val-Leu-Cys-Gly-Gly-Glu-Trp-Trp-His-Cys-Cys-Gly-Leu-Gly-
Cys-Gly-Trp-Val-Val-Glu-Ala-Cys (SEQ ID NO: 50)
Cys-Ser-Trp-Val-Ser-Val-Leu-Cys-Gly-Gly-Glu-Trp-Trp-Gln-Cys-Cys-Gly-Pro-Gly-
Cys-Gly-Leu-Val-Val-Asn-Ala-Cys (SEQ ID NO: 51)

5 Cys-Ser-Trp-Val-Ser-Leu-Gln-Cys-Gly-Gly-Glu-Trp-Trp-His-Cys-Cys- Gly-Gly-Gly-
Cys-Gly-Trp-Val-Val-Asn-Val-Cys (SEQ ID NO: 52)
Cys-Ser-Trp-Val-Leu-Leu-His-Cys-Gly-Gly-Glu-Trp-Trp-His-Cys-Cys-Gly-Gly-Gly-
Cys-Gly-Trp-Val-Gly-Glu-Ala-Cys (SEQ ID NO: 53)
Cys-Ser-Trp-Val-Leu-Leu-Glu-Cys-Gly-Gly-Glu-Leu-Trp-Glu-Cys-Cys Gly-Leu-Gly-

10 Cys-Gly-Trp-Val-Ala-Asp-Ala-Cys (SEQ ID NO: 54)
Cys-Ser-Trp-Val-Val-Phe-Glu-Cys-Leu-Gly-Glu-Ser-Trp-His-Cys-Cys-Gly-Gly-Gly-
Cys-Gly-Trp-Val-Val-His-Ala-Cys (SEQ ID NO: 55)
Cys-Ala-Trp-Val-Ser-Val-Glu-Cys-Gly-Gly-Glu-Trp-Trp-His-Cys-Cys-Gly-Pro-Gly-
Cys-Gly-Trp-Val-Val-Asp-Ala-Cys (SEQ ID NO: 56)

15   Peptide Ligand Combinations

         A.   Multimerization domains

         According to a preferred embodiment of the invention, the peptide ligands
are combined with a multimerization domain. According to this aspect of the
invention, hybrid molecules are provided which comprise at least two distinct
20   domains. Each molecule comprises a peptide ligand domain and a multimerization
domain. According to the present invention, the peptide ligand domain is
joined to a multimerization domain such as an immunoglobulin Fc region,
optionally via a flexible linker domain.

         The hybrid molecules of the present invention are constructed by
25   combining the peptide ligands with a suitable multimerization domain.
Ordinarily, when preparing the hybrid molecules of the present invention,
nucleic acid encoding the peptide ligand will be operably linked to nucleic
acid encoding the multimerization domain sequence. Typically the construct
encodes a fusion protein wherein the C-terminus of the peptide ligand is joined
30   to   the   N-terminus   or   C-terminus,   preferably   the   C-terminus   of   the
multimerization domain. However, fusions where, for example, the N-terminus
of the peptide ligand is joined to the N-terminus or C-terminus of the
multimerization domain are also possible.

         Preferred multimerization domains are immunoglobulin constant region
35   sequences. Typically, in such fusions the encoded hybrid molecule will retain
at least functionally active hinge, CH2 and CH3 domains of the constant region
of an immunoglobulin heavy chain. Fusions are also made, for example, to the
C-terminus of the Fc portion of a constant domain, or immediately N-terminal
to the CH1 of the heavy chain or the corresponding region of the light chain.

40   The precise amino acid site at which the fusion of the peptide ligand to
the immunoglobulin constant domain is made is not critical; particular sites
are well known and may be selected in order to optimize the biological
activity, secretion, or binding characteristics. In this regard, the skilled

artisan may reference the construction of various immunoadhesins described in the literature (U.S. Patent No.s 5,116,964, 5,714,147 and 5,336,603; Capon et al., (1989) Nature 337:525-531; Traunecker et al., (1989) Nature 339:68-70; and Byrn et al., (1990) Nature 344:667-670; Watson et al., (1990) J. Cell. Biol. 110:2221-2229; Watson et al., (1991) Nature 349:164-167; Aruffo et al., (1990) Cell 61:1303-1313; Linsley et al., (1991) J. Exp. Med. 173:721-730; Lisley et al., J. Exp. Med. 174:561-569; Stamenkovic et al., Cell 66:1133-1144; Ashkenazi et al., (1991) Proc. Natl. Acad. Sci. USA 88:10535-10539; Lesslauer et al., (1991) Eur. J. Immunol. 27:2883-2886; and Peppel et al., (1991) J. Exp. Med. 174:1483-1489; Mohler et al., (1993) J. Immunol. 151:1548-1561); Bennett et al., (1991) J. Biol. Chem. 266:23060-23067; Kurschner et al., (1992) J. Biol. Chem. 267:9354-9360; Chalupny et al., (1992) PNAS USA 89:10360-10364; Ridgway and Gorman, (1991) J. Cell. Biol. 115, Abstract No. 1448).

According to a particular aspect, an immunoglobulin type multimerization domain is selected to provide a multimer such as a dimer having a functional Fc. In preferred aspects the multimerization domain is selected to provide an Fc domain having an effector function associated with a native immunoglobulin Fc region. Therefore, the peptide ligand is joined, in particular aspects, to an immunoglobulin heavy chain constant domain to provide a multimer comprising a functional Fc domain selected for a particular effector function or functions. In this case, DNA encoding an immunoglobulin chain-peptide ligand sequence is typically coexpressed with the DNA encoding a second peptide ligand-immunoglobulin heavy chain fusion protein. Upon secretion, the hybrid heavy chain will be covalently associated to provide an immunoglobulin-like structure comprising two disulfide-linked immunoglobulin heavy chains. The skilled artisan will recognize that effector functions include, for example, C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; and down regulation of cell surface receptors (e.g. B cell receptor; BCR) and prolonging half-life through incorporation of the salvage receptor binding ligand as described in, for example, U.S. patent no. 5,739,277 issued April 14, 1998.

Preferably, the Fc region is a human Fc region, e.g. a native sequence human Fc region human IgG1 (A and non-A allotypes), IgG2, IgG3 or IgG4 Fc region. Such sequences are shown in Figures 2 & 3. Additionally, by introducing the appropriate amino acid sequence modifications in a parent Fc region, one can generate a variant Fc region which (a) mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of human effector cells more effectively and/or (b) binds an Fc gamma receptor (Fc(R) with better affinity than the native sequence. Such Fc region variants will generally comprise at least one amino acid modification in the Fc region.

In a preferred embodiment, the peptide ligand sequence is fused to the N-terminus of the Fc region of immunoglobulin G_1 (IgG_1). It is possible to fuse the entire heavy chain constant region to the peptide ligand sequence. However, more preferably, a sequence beginning in the hinge region just

upstream of the papain cleavage site which defines IgG Fc chemically (i.e. residue 216, taking the first residue of heavy chain constant region to be 114), or analogous sites of other immunoglobulins is used in the fusion. In a particularly preferred embodiment, the peptide ligand amino acid sequence is fused to (a) the hinge region (or other linker domain) and CH2 and CH3 or (b) the CH1, hinge, CH2 and CH3 domains, of an IgG heavy chain. In a further preferred embodiment the peptide ligand amino acid sequence is fused to (a) the hinge region (or other linker domain) and (b) the CH3 domain of an IgG1 (see, for example, the constructs described in Hu et al., (1996) Cancer Res. 56:3055-3061).

According to a particular aspect of this embodiment, hybrid molecules comprising a peptide ligand and a multimerization domain are assembled as multimers, for example homodimers, or heterodimers or even heterotetramers. Homodimers result from the pairing or crosslinking of two monomers comprising a peptide ligand and a multimerization domain. However, it is not essential that two identical monomers pair. According to a particular aspect of the invention a hybrid molecule as defined herein comprising a peptide ligand and a multimerization domain such as an immunoglobulin constant domain may pair with a companion immunoglobulin chain comprising one arm of an immunoglobulin. Various exemplary assembled hybrid molecules within the scope of the present invention are schematically diagramed below:

     (a) ACH

     (b) ACH-ACH

     (c) ACH-VHCH-VLCL

     (d) ACH-VHCH

wherein each A represents identical or different peptide ligands;

VL is an immunoglobulin light chain variable domain;

VH is an immunoglobulin heavy chain variable domain;

CL is an immunoglobulin light chain constant domain and

CH is an immunoglobulin heavy chain constant domain.

In the interests of brevity, the foregoing structures only show key features; they do not show optional linker domains between the peptide ligand domains and the multimerization domains as described herein below; they do not indicate joining, hinge or other domains of the immunoglobulins, nor are disulfide bonds shown. However, where such domains are required for binding activity, they are constructed to be present in the ordinary locations which they occupy in the immunoglobulin molecules.

Although the presence of an immunoglobulin light chain is not required in the hybrid molecules of the present invention, an immunoglobulin light chain might be present either covalently associated to a peptide ligand-immunoglobulin heavy chain fusion polypeptide, or directly fused to the peptide ligand. In the former case, DNA encoding an immunoglobulin light chain is typically coexpressed with the DNA encoding the peptide ligand-immunoglobulin heavy chain fusion protein. Upon secretion, the hybrid heavy chain and the

light chain will be covalently associated to provide an immunoglobulin-like structure comprising two disulfide-linked immunoglobulin heavy chain-light chain pairs.

The hybrid molecules described herein are most conveniently constructed by fusing the cDNA sequence encoding the peptide ligand portion in-frame to an immunoglobulin cDNA sequence. However, fusion to genomic immunoglobulin fragments can also be used (see, e.g. Aruffo et al., (1990), Cell 61:1303-1313; and Stamenkovic et al. (1991), Cell 66:1133-1144). The latter type of fusion requires the presence of Ig regulatory sequences for expression. cDNAs encoding IgG heavy-chain constant regions can be isolated based on published sequences from cDNA libraries derived from spleen or peripheral blood lymphocytes, by hybridization or by polymerase chain reaction (PCR) techniques. The cDNAs encoding the peptide ligand and the immunoglobulin parts of the hybrid molecule are inserted in tandem into a plasmid vector that directs efficient expression in the chosen host cells.

Alternatively, and especially in embodiments where the peptide ligand is synthesized by, for example standard solid phase synthesis techniques, the peptide ligand may be linked to the multimerization domain by any of a variety of means familiar to those of skill in the art. Covalent attachment is typically the most convenient, but other forms of attachment may be employed depending upon the application. Examples of suitable forms of covalent attachment include the bonds resulting from the reaction of molecules bearing activated chemical groups with amino acid side chains in the multimerization domain and can be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene).

The invention includes the specific targeting of, for example HER2 bearing cell types, in vivo via the peptide ligands and according to this aspect of the present invention, conferring an Fc effector funtion such as Fc receptor binding, complement fixation, protein A binding, increasing half-life or crossing the blood brain barrier to the molecule.

B. Peptide Ligand Fusions

According to the present invention, the peptide ligand domain is optionally linked to, for example, another peptide ligand domain either directly or via a flexible peptide linker as described below. According to the present invention, the linker domain, is any group of molecules that provides a spatial bridge between two or more peptide ligand domains as described in more detail herein below. According to this aspect of the invention, peptide ligands are linked together, as for example in a fusion protein. The hybrid

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molecules of this aspect of the invention are useful in, for example, cross-linking two or more receptors.

C. Linker Domains

According to the present invention, the peptide ligand domain is optionally linked to, for example, another peptide ligand domain or a multimerization domain via a flexible peptide linker. The linker component of the hybrid molecule of the invention does not necessarily participate in but may contribute to the function of the hybrid molecule. Therefore, according to the present invention, the linker domain, is any group of molecules that provides a spatial bridge between two or more peptide ligand domains or a peptide ligand domain and a mulitmerization domain.

The linker domain can be of variable length and makeup. It is generally, the orientation caused by the linker domain and not its chemical structure that is important. The linker domain preferably allows for the peptide ligand domain of the hybrid molecule to bind, substantially free of spacial/conformational restrictions to the coordinant ErbB2 molecule. Therefore, the length of the linker domain is dependent upon the character of the two functional, e.g., the peptide ligand and the multimerization domains of the hybrid molecule.

One skilled in the art will recognize that various combinations of atoms provide for variable length molecules based upon known distances between various bonds (Morrison, and Boyd, Organic Chemistry, 3rd Ed, Allyn and Bacon, Inc., Boston, MA (1977)). For example, the linker domain may be a polypeptide of variable length. The amino acid composition of the polypeptide determines the character and length of the linker. Exemplary, linker domains comprises between 2 and 10 amino acids, preferably about 6 amino acids as, for example, Gly-Gly-Gly-Ser-Gly-Gly (SEQ ID NO:129), Gly-Gly-Gly-Ser-Ser-Gly (SEQ ID NO:130), and Gly-Gly-Gly-Arg-Gly-Gly (SEQ ID NO:131).

D. Other Bispecific Combinations

According to certain aspects of the invention, bi- or dual-specific compositions comprising at least one peptide ligand domain are envisioned. For example, bispecific antibody compositions have been produced using leucine zippers (Kostelny et al., (1992) J. Immunol., 148(5):1547-1553). The leucine zipper peptides from the Fos and Jun proteins are linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers can be reduced at the hinge region to form monomers and then re-oxidized to form antibody heterodimers. This method can also be utilized for the production of peptide ligand homodimer and heterodimers utilizing the peptide ligands in place of the binding domains of the antibody heterodimers.

Recombinant Synthesis

The present invention encompasses a composition of matter comprising isolated nucleic acid, preferably DNA, encoding a peptide described herein. DNAs encoding the peptides of the invention can be prepared by a variety of methods known in the art. These methods include, but are not limited to,

chemical synthesis by any of the methods described in Engels et al., (1989)
Agnew. Chem. Int. Ed. Engl., 28:716-734, the entire disclosure of which is
incorporated herein by reference, such as the triester, phosphite,
phosphoramidite and H-phosphonate methods. In one embodiment, codons preferred
5   by the expression host cell are used in the design of the encoding DNA.
Alternatively, DNA encoding the peptide can be altered to encode one or more
variants by using recombinant DNA techniques, such as site specific mutagenesis
(Kunkel et al., (1991) Methods Enzymol. 204:125-139; Carter, P., et al., (186)
Nucl. Acids. Res. 13:4331; Zoller, M. J. et al., (1982) Nucl. Acids Res.
10   10:6487), cassette mutagenesis (Wells, J. A., et al., (1985) Gene 34:315),
restriction selection mutagenesis (Wells, J. A., et al., (1986) Philos. Trans,
R. Soc. London SerA 317, 415), and the like.

        The invention further comprises an expression control sequence operably
linked to the DNA molecule encoding a peptide of the invention, and an
15   expression vector, such as a plasmid, comprising the DNA molecule, wherein the
control sequence is recognized by a host cell transformed with the vector. In
general, plasmid vectors contain replication and control sequences which are
derived from species compatible with the host cell. The vector ordinarily
carries a replication site, as well as sequences which encode proteins that are
20   capable of providing phenotypic selection in transformed cells.

        Suitable host cells for expressing the DNA include prokaryote, yeast, or
higher eukaryote cells. Suitable prokaryotes include but are not limited to
eubacteria, such as Gram-negative or Gram-positive organisms, for example,
Enterobacteriaceae such as E. coli. Various E. coli strains are publicly
25   available, such as E. coli K12 strain MM294 (ATCC 31,446); E. coli X1776 (ATCC
31,537); E. coli strain W3110 (ATCC 27,325) and K5 772 (ATCC 53,635).

        In addition to prokaryotes, eukaryotic organisms, such as yeasts, or
cells derived from multicellular organisms can be used as host cells. For
expression in yeast host cells, such as common baker's yeast or Saccharomyces
30   cerevisiae, suitable vectors include episomally replicating vectors based on
the 2-micron plasmid, integration vectors, and yeast artificial chromosome
(YAC) vectors. Suitable host cells for expression also are derived from
multicellular organisms. Examples of invertebrate cells include insect cells
such as Drosophila S2 and Spodoptera Sf9, as well as plant cells. For
35   expression in insect host cells, such as Sf9 cells, suitable vectors include
baculoviral vectors. For expression in plant host cells, particularly
dicotyledonous plant hosts, such as tobacco, suitable expression vectors
include vectors derived from the Ti plasmid of Agrobacterium tumefaciens.

        Examples of useful mammalian host cells include monkey kidney CV1 line
40   transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293
or 293 cells subcloned for growth in suspension culture, Graham et al., (1977)
J. Gen Virol., 36:59); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese
hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, (1980) Proc. Natl. Acad.
Sci. USA, 77:4216); mouse sertoli cells (TM4, Mather, (1980) Biol. Reprod.,

23:243-251); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep

5   G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., (1982) Annals N.Y. Acad. Sci., 383:44-68); MRC 5 cells; FS4 cells; and a human hepatoma cell line (Hep G2).

For expression in prokaryotic hosts, suitable vectors include pBR322 (ATCC No. 37,017), phGH107 (ATCC No. 40,011), pBO475, pSO132, pRIT5, any vector

10   in the pRIT20 or pRIT30 series (Nilsson and Abrahmsen, (1990) Meth. Enzymol., 185:144-161), pRIT2T, pKK233-2, pDR540 and pPL-lambda. Prokaryotic host cells containing the expression vectors of the present invention include *E. coli* K12 strain 294 (ATCC NO. 31446), *E coli* strain JM101 (Messing et al.,(1981) Nucl.Acid Res., 9:309), *E. coli* strain B, *E. coli* strain χ1776 (ATCC No.

15   31537), *E. coli* c600 (Appleyard, *Genetics*, 39: 440 (1954)), *E. coli* W3110 (F-, gamma-, prototrophic, ATCC No. 27325), *E. coli* strain 27C7 (W3110, *tonA, phoA E15, (argF-lac)169, ptr3, degP41, ompT, kan^r*) (U.S. Patent No. 5,288,931, ATCC No. 55,244), *Bacillus subtilis, Salmonella typhimurium, Serratia marcesans,* and *Pseudomonas* species.

20   For expression in mammalian host cells, useful vectors include vectors derived from SV40, vectors derived from cytomegalovirus such as the pRK vectors, including pRK5 and pRK7 (Suva et al., (1987) Science, 237:893-896; EP 307,247 (3/15/89), EP 278,776 (8/17/88)) vectors derived from vaccinia viruses or other pox viruses, and retroviral vectors such as vectors derived from

25   Moloney's murine leukemia virus (MoMLV).

Optionally, the DNA encoding the peptide of interest is operably linked to a secretory leader sequence resulting in secretion of the expression product by the host cell into the culture medium. Examples of secretory leader sequences include stII, ecotin, lamB, herpes GD, lpp, alkaline phosphatase,

30   invertase, and alpha factor. Also suitable for use herein is the 36 amino acid leader sequence of protein A (Abrahmsen et al., (1985) EMBO J., 4:3901).

Host cells are transfected and preferably transformed with the above-described expression or cloning vectors of this invention and cultured in conventional nutrient media modified as appropriate for inducing promoters,

35   selecting transformants, or amplifying the genes encoding the desired sequences.

Transfection refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, for

40   example, CaPO$_4$ precipitation and electroporation. Successful transfection is generally recognized when any indication of the operation of this vector occurs within the host cell.

Transformation means introducing DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in section 1.82 of Sambrook et al., Molecular Cloning (2nd ed.), Cold Spring Harbor Laboratory, NY (1989), is generally used for prokaryotes or other cells that contain substantial cell-wall barriers. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw et al., (1983) Gene, 23:315 and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method described in sections 16.30-16.37 of Sambrook et al., supra, is preferred. General aspects of mammalian cell host system transformations have been described by Axel in U.S. 4,399,216 issued 16 August 1983. Transformations into yeast are typically carried out according to the method of Van Solingen et al., (1977) J. Bact., 130:946 and Hsiao et al., (1979) Proc. Natl. Acad. Sci. (USA), 76:3829. However, other methods for introducing DNA into cells such as by nuclear injection, electroporation, or by protoplast fusion may also be used.

Other preferred vectors can be constructed using standard techniques by combining the relevant traits of the vectors described above. Relevant traits include the promoter, the ribosome binding site, the gene of interest or gene fusion (the Z domain of protein A and the gene of interest and a linker), the antibiotic resistance markers, and the appropriate origins of replication.

A variation on the above procedures contemplates the use of gene fusions, wherein the gene encoding the desired peptide is associated, in the vector, with a gene encoding another protein or a fragment of another protein. This results in the desired peptide being produced by the host cell as a fusion with another protein or peptide. The "other" protein or peptide is often a protein or peptide which can be secreted by the cell, making it possible to isolate and purify the desired peptide from the culture medium and eliminating the necessity of destroying the host cells which arises when the desired peptide remains inside the cell. Alternatively, the fusion protein can be expressed intracellularly. It is useful to use fusion proteins that are highly expressed.

The use of gene fusions, though not essential, can facilitate the expression of heterologous peptides in insect cells as well as the subsequent purification of those gene products. Protein A fusions are often used because the binding of protein A, or more specifically the Z domain of protein A, to IgG provides an "affinity handle" for the purification of the fused protein. For example, a DNA sequence encoding the desired peptide ligand can be fused by site directed mutagenesis to the gene for a consensus domain of protein A known as the Z domain (Nilsson et al., (1987) Protein Engineering 1:107-113). After expression and secretion the fusion protein can be enzymatically cleaved to yield free peptide which can be purified from the enzymatic mix (see, e.g.,

Varadarajan et al., (1985) Proc. Natl. Acad. Sci USA 82:5681-5684; Castellanos-
Serra et al., (1996) FEBS Letters 378:171-176; Nilsson et al., (1996) J.
Biotechnol. 48:241-250).

5   Fusion proteins can be cleaved using chemicals, such as cyanogen bromide,
which cleaves at a methionine, or hydroxylamine, which cleaves between an Asn
and Gly residue. Using standard recombinant DNA methodology, the nucleotide
base pairs encoding these amino acids may be inserted just prior to the 5' end
of the gene encoding the desired peptide.

       Alternatively, one can employ proteolytic cleavage of fusion protein.
10   Carter, in Protein Purification: From Molecular Mechanisms to Large-Scale
Processes, Ladisch et al., eds. (American Chemical Society Symposium Series
No. 427, 1990), Ch 13, pages 181-193.

       Proteases such as Factor Xa, thrombin, and subtilisin or its mutants, and
a number of others have been successfully used to cleave fusion proteins.
15   Preferred according to the present invention for the production of peptide
ligands of less than about 30 amino acids is the protease trypsin which is
highly specific for Arg and Lys residues. Trypsin cleavage is discussed
generally in Nilsson et al. (1996) J. Biotech. 48:241 and Smith et al., Methods
Mol. Biol. 32:289. Typically, a peptide linker that is amenable to cleavage
20   by the protease used is inserted between the "other" protein (e.g., the Z
domain of protein A) and the desired peptide. Using recombinant DNA
methodology, the nucleotide base pairs encoding the linker are inserted between
the genes or gene fragments coding for the other proteins. Proteolytic
cleavage of the partially purified fusion protein containing the correct linker
25   can then be carried out on either the native fusion protein, or the reduced or
denatured fusion protein.

       The peptide may or may not be properly folded when expressed as a fusion
protein. Also, the specific peptide linker containing the cleavage site may
or may not be accessible to the protease. These factors determine whether the
30   fusion protein must be denatured and refolded, and if so, whether these
procedures are employed before or after cleavage.

       When denaturing and refolding are needed, typically the peptide is
treated with a chaotrope, such a guanidine HCl, and is then treated with a
redox buffer, containing, for example, reduced and oxidized dithiothreitol or
35   glutathione at the appropriate ratios, pH, and temperature, such that the
peptide is refolded to its native structure.

       The host cells referred to in this disclosure encompass cells in in vitro
culture as well as cells that are within a host animal.

       In cyclized embodiments of the invention, the recombinantly produced
40   peptide can be cyclized by formation of an intramolecular disulfide bond as
described above.
Utility

       Generally speaking, the peptide ligands and hybrid molecules of the
present invention can be used in the same applications as, for example, native

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or variant heregulin molecules or antibodies directed against ErbB2 may be used. Of course, some peptide ligands or hybrid molecules within the scope of the present invention may be better suited for a particular application than another application. Those skilled in the art will readily ascertain which molecules are appropriate for a given application by using one or more conventional biological assay to determine the biological activity of the peptide ligand or hybrid molecule.

As but an example, heregulins are useful in the treatment of a variety of disorders, for example, disorders and diseases affecting the nervous system, musculature and epithelia. For example, an agonist peptide ligand or hybrid molecule of the present invention may be used in promoting the development, maintenance and or regeneration of a neuron _in vivo_ in the same manner that heregulin or a heregulin variant may be used. Diseases or disorders amenable to treatment with peptide ligands or hybrid molecules according to this aspect of the invention include, for example, central nervous system damage attendant to trauma, surgery, stroke, ischemia, infection, metabolic disorders nutritional deficiency, malignancy or a toxic agent. Neurodegenerative disorders my also be treated included but not limited to human neurodegenerative diseases or disorders such as Alzheimer's disease, Parkinson's disease, epilepsy, multiple sclerosis, Huntington's chorea, Downs syndrome, nerve deafness and Meniere's disease. As well, particular peptide ligands are suited for use in the treatment of neuropathy, such as peripheral neuropathy associated with systemic diseases or disorders such as diabetes.

Agonist peptide ligands which upon binding ErbB2 initiate a phosphorylation event as described are especially useful the foregoing aspects of the invention. Specific agonist peptide ligands of the invention are capable of enhancing the survival of cells, which is to say they increase the period of survival of an ErbB2 bearing cell either _in vivo_ or _in vitro_ relative to the period of survival of cells that have not been exposed to a particular agonist peptide ligand. In preferred embodiments, the agonist peptide ligand causes or enhances the proliferation of ErbB2 bearing cell types, either _in vivo_ or _in vitro_, as quantified by, for example, measuring ^{3}H-thymidine uptake by cells. Proliferation may be associated with differentiation of ErbB2 cells as determined by screening an ErbB2 bearing cell population for phenotypic changes.

According to another aspect of the present invention, certain peptide ligands and hybrid molecules, especially those that bind ErbB2 but do not elicit an agonist associated response, are useful in, for example, inhibiting tumor cell invasion and metastasis as described more fully herein below. For example, a tumor that expresses ErbB2 receptors (especially one which overexpresses ErbB2) can be treated using a peptide ligand or hybrid molecule conjugated to a cytotoxic agent or in targeting a prodrug to cells expressing ErbB2.

A.   Effector Function Engineering

For example, it may be desirable to modify the multimerization domain of hybrid molecules of the present invention with respect to effector function, to, for example, enhance the effectiveness of the antibody in treating cancer. For example, cysteine residue(s) may be introduced in an immunoglobulin Fc region, thereby allowing interchain disulfide bond formation in this region. The homo- or heterodimeric hybrid molecule thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron et al., (1992) J. Exp Med. 176:1191-1195 and Shopes, B. (1992) J. Immunol. 148:2918-2922. Homodimeric hybrid molecules with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff et al. (1993) Cancer Research 53:2560-2565.

Alternatively, an heterodimeric hybrid molecule can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson et al. (1989) Anti-Cancer Drug Design 3:219-230.

B.   Conjugates

The invention also pertains to conjugates comprising the peptide ligands or any of the hybrid molecules described herein conjugated to a cytotoxic agent such as a chemotherapeutic agent, such as a protein toxin or cytotoxic drug or toxin (e.g. an enzymatically active toxin of bacterial, fungal, plant or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described. A chemotherapeutic agent is a chemical compound useful in the treatment of cancer including carcinoma, lymphoma, blastoma, sarcoma, and leukemias. Examples of chemotherapuetic agents include, antibiotics isolated from microorganisms such as the calicheamicins (Lee et al., (1987) J. Am. Chem. Soc. 109:3464-3466; Hinman et al., (1993) Cancer Res. 53:3336-3342), maytansinoids (such as those described in Liu et al. (1996) Proc. Natl. Acad. Sci. USA 93:8618-8323), Adriamycin, Doxorubicin, 5-Fluorouracil, Cytosine arabinoside, Cyclophophamide, thiotepa, Busulfan, Cytoxin, Taxol, Methotrexate, Cisplatin, Melphalan, Vinblastin, Bleomycin, Etoposide, Ifosfamide, Mitomycin C, Mitosantrone, Vincristine, Vinorelbine, Carboplatin, Teniposide, Daunomycin, Carminomydcin, Aminopterin, Dactinomycin, a Mitomycin, Nicotinamide, an Espeeramicin, Melphalan and any related nitrogen mustard, and endocrine therapeutics (such as diethylstilbestrol, Tamoxifen, LHRH-antagonizing drugs, a progestin, an anti-progestin, etc.)

Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia

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inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes.

A variety of radionuclides are available for the production of radioconjugated peptide ligands or hybrid molecules. Examples include ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y and ^{186}Re. Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the hybrid molecules.

Conjugates of the peptide ligand or hybrid molecule and a cytotoxic agent are made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared essentially as described in Vitetta et al. (1987) Science 238: 1098.

In another embodiment, the peptide ligand or hybrid molecules may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the peptide ligand or hybrid molecule-receptor conjugate is administered to a patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g. avidin) which is conjugated to a cytotoxic agent (e.g. a radionucleotide).

C. Liposomes

The hybrid molecules or peptide ligands disclosed herein may also be formulated as liposomes. Liposomes containing the hybrid molecules are prepared by methods known in the art, such as described in Epstein et al., (1985) Proc. Natl. Acad. Sci. USA, 82:3688; Hwang et al., (1980) Proc. Natl Acad. Sci. USA, 77:4030; and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. For example, hybrid molecules comprising an immunoglobulin constant domain as described herein can be conjugated to the liposomes as described in Martin et al. J. Biol. Chem. 257: 286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent is optionally contained within the liposome. See Gabizon et al. J. National Cancer Inst.81(19)1484 (1989).

D. Enzyme Mediated Prodrug Therapy

The peptide ligands or hybrid molecules of the present invention may also be used in the manner of antibodies of antibody dependent enzyme mediated prodrug therapy (ADEPT) by conjugating the peptide ligand or hybrid molecules

to a prodrug-activating enzyme which converts a prodrug (e.g. a peptidyl chemotherapeutic agent, see WO81/01145) to an active anti-cancer drug. See, for example, WO 88/07378 and U.S. Patent No. 4,975,278.

The enzyme component of the conjugate useful for ADEPT-type therapy includes any enzyme capable of acting on a prodrug in such a way so as to covert it into its more active, cytotoxic form. Enzymes that are useful in the method of this invention include, but are not limited to, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as β-galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs; β-lactamase useful for converting drugs derivatized with β-lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes", can be used to convert the prodrugs of the invention into free active drugs (see, e.g., Massey, (1987) Nature 328:457-458). Peptide ligand/hybrid molecule-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a tumor cell population.

Enzymes can be covalently bound to the hybrid molecules by techniques well known in the art such as the use of the heterobifunctional crosslinking reagents discussed above. Alternatively, fusion proteins comprising at least the peptide ligand portion of the hybrid molecule of the invention linked to at least a functionally active portion of an enzyme of the invention can be constructed using recombinant DNA techniques well known in the art (see, e.g., Neuberger et al., (1984) Nature, 312:604-608).

E. Pharmaceutical Compositions

Pharmaceutical compositions which comprise the peptide ligands of the invention may be administered in any suitable manner, including parental, topical, oral, or local (such as aerosol or transdermal) or any combination thereof. Suitable regimens also include an initial administration by intravenous bolus injection followed by repeated doses at one or more intervals.

Pharmaceutical compositions of the compounds of the invention are prepared for storage by mixing a peptide ligand containing compound having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. [1980]), in the form of lyophilized formulations or aqueous

solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium

5   chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as

10   polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes);

15   and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

The compositions herein may also contain more than one active compounds as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example,

20   it may be desirable to further provide molecules, such as antibodies which bind to EGFR, ErbB2 (e.g. an antibody which binds a different ligand on ErbB2), ErbB3, ErbB4, or vascular endothelial factor (VEGF) in the one formulation. Alternatively, or in addition, the composition may comprise a cytotoxic agent, cytokine, growth inhibitory agent and/or cardioprotectant. Such molecules are

25   suitably present in combination in amounts that are effective for the purpose intended.

F.   Diagnosing and Prognosing a Disorder

Disorders for prognosis and diagnosis within the context of the present invention are preferably a benign or malignant tumor characterized by the

30   overexpression of the ErbB2 receptor, e.g. a cancer, such as, breast cancer, squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, colon cancer, colorectal cancer, endometrial carcinoma, salivary gland carcinoma, kidney

35   cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer. Therefore, one embodiment of the present invention is directed to the detection and/or measurement of Erb2 in a sample and the use of such detection or measurement in the diagnosis, staging, determination of severity, and prognosis in general of the disorder.

40   In a particular embodiment, the diagnostic techniques described can be used to follow the progress of therapy. In a subject undergoing therapeutic treatment that results in an increase or a decrease in the amount of Erb2 bearing cells, the amount of Erb2 bearing cells in a sample may serve as a useful measure for the success or failure of the treatment. Thus, the present

invention provides a method for monitoring the effect of a therapeutic treatment in a subject which comprises measuring at suitable time intervals the amount of Erb2 expressed in a sample of tissue suspected of containing Erb-2 expressing cells. The total amount of Erb2 is compared to a "baseline" or
5   "control" value which depending on the disease, and the treatment, may be the amount of Erb2 in a similar sample from a normal subject, from the patient prior to disease onset or during remission of disease, or from the patient prior to the initiation of therapy. One of ordinary skill in the art will readily discern the appropriate baseline value to use in a particular situation
10   without undue experimentation.

Any procedure known in the art for the measurement of analytes can be used in the practice of the measurement of Erb2 in a sample using the compounds of the present invention. Such procedures include but are not limited to competitive and non-competitive assay systems using techniques such
15   as radioimmunoassays, enzyme immunoassays (EIA), preferably the enzyme linked immunosorbent assay (ELISA), "sandwich" immunoassays, precipitin reactions, gel diffusion reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, and immunoelectrophoresis assays, to name but a few.
20   For examples of preferred immunoassay methods, see U.S. Patents No. 4,845,026 (July 4, 1989) and No. 5,006,459 (April 9, 1991).

For diagnostic and prognostic applications, a compound of the present invention, typically a hybrid molecule as described herein above will be labeled with a detectable moiety and used to detect Erb-2 in a sample as
25   described above. Numerous labels are available which can be preferably grouped into the following categories:

(a) Radioisotopes, such as ^{35}S, ^{14}C, ^{125}I, ^{3}H, and ^{131}I. The hybrid molecules can be labeled with the radioisotope using the techniques described in *Current Protocols in Immunology*, Volumes 1 and 2, Coligen *et al.*, Ed.,
30   Wiley-Interscience, New York, New York, Pubs., (1991) for example and radioactivity can be measured using scintillation counting.

(b) Fluorescent labels such as rare earth chelates (europium chelates) or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, Lissamine, phycoerythrin and Texas Red are available. The fluorescent labels
35   can be conjugated to the hybrid molecules using the techniques disclosed in *Current Protocols in Immunology, supra*, for example. Fluorescence can be quantified using a fluorimeter.

(c) Various enzyme-substrate labels are available and U.S. Patent No. 4,275,149 provides a review of some of these. The enzyme preferably catalyses
40   a chemical alteration of the chromogenic substrate which can be measured using various techniques. For example, the enzyme may catalyze a color change in a substrate, which can be measured spectrophotometrically. Alternatively, the enzyme may alter the fluorescence or chemiluminescence of the substrate.

Techniques for quantifying a change in fluorescence are described above. The chemiluminescent substrate becomes electronically excited by a chemical reaction and may then emit light which can be measured (using a chemiluminometer, for example) or donates energy to a fluorescent acceptor.

5 Examples of enzymatic labels include luciferases (e.g., firefly luciferase and bacterial luciferase; U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase such as horseradish peroxidase (HRPO), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases (e.g., glucose oxidase, galactose

10 oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (such as uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like. Techniques for conjugating enzymes to antibodies are described in O'Sullivan et al., Methods for the Preparation of Enzyme-Antibody Conjugates for use in Enzyme Immunoassay, in Methods in Enzym. (ed J. Langone & H. Van

15 Vunakis), Academic press, New York, 73: 147-166 (1981).

Examples of enzyme-substrate combinations include, for example:

(i)   Horseradish peroxidase (HRPO) with hydrogen peroxidase as a substrate, wherein the hydrogen peroxidase oxidizes a dye precursor (e.g. orthophenylene diamine (OPD) or 3,3',5,5'-tetramethyl benzidine hydrochloride

20 (TMB));

(ii)   alkaline phosphatase (AP) with para-Nitrophenyl phosphate as chromogenic substrate; and

(iii) β-D-galactosidase (β-D-Gal) with a chromogenic substrate (e.g. p-nitrophenyl-β-D-galactosidase) or fluorogenic substrate 4-methylumbelliferyl-β-

25 D-galactosidase.

Numerous other enzyme-substrate combinations are available to those skilled in the art. For a general review of these, see U.S. Patent Nos. 4,275,149 and 4,318,980.

In the assays of the present invention, a hybrid molecule is preferably

30 bound to a solid phase support or carrier. By "solid phase support or carrier" is intended any support capable of binding an antigen or antibodies. Well-known supports, or carriers, include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amyloses, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be

35 either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the

40 external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

In a preferred embodiment, a sandwich immunoassay is employed, *i.e.*, Erb2 is detected or measured by a method comprising binding of a first antibody or hybrid molecule to the Erb2 antigen, and binding of a second antibody or hybrid molecule to the Erb2, and detecting or measuring Erb2 immunospecifically bound

5   by both the first and second antibody or hybrid molecule. In a specific embodiment, either the first or second antibodies are monoclonal antibodies and either or both of the first and second are hybrid molecules of the present invention. In this embodiment, the peptide ligand portion of the hybrid molecule preferably binds to a site different from that of the first antibody

10   (as reflected *e.g.*, by the lack of competitive inhibition between the antibody and the hybrid molecule for binding to the antigen). In another specific embodiment, the first or second antibody is a polyclonal antibody.

       Kits comprising one or more containers or vials containing components for carrying out the assays of the present invention are also within the scope of

15   the invention. For instance, such a kit can comprise reagents required for the immunohistochemical analysis of a sample such as a tumor biopsy. Reagents may include one or more binding partners, *e.g.* a hybrid molecule or an antibody. For histological assays the kit contains the chromogenic substrate as well as a reagent for stopping the enzymatic reaction when color development has

20   occurred. The substrate included in the kit is one appropriate for the enzyme conjugated to one of the hybrid molecules of the present invention. These are well-known in the art. The kit can optionally also comprise a standard, *e.g.*, a known amount of purified Erb2.

       The following examples are offered by way of illustration and not by way

25   of limitation. The disclosures of all citations in the specification are expressly incorporated herein by reference.

EXAMPLES

EXAMPLE I

Identification and Maturation of HER2 Binding Peptides

30   Methods

Phage Libraries- Random sequence polyvalent peptide phage libraries (**Table 1**) were constructed using large scale Kunkel mutagenesis of the template pB2479.g8. This phagemid contains the Tac promoter the mal signal sequence, 3 stop codons and a linker sequence fused to gVIII and includes the $LacI^q$ and

35   ampicillin resistance genes. The peptide libraries are thus expressed polyvalently on phage as fusions to pVIII with the ability to regulate copy number with IPTG. Each library has a diversity in excess of 10^9 clones.

Table 1

	Sequence
Pool 1	$X_4CX_2GPX_4CX_4$ $X_7CX_4CX_7$ $X_7CX_5CX_6$ $X_6CX_6CX_6$
Pool 2	$X_6CX_7CX_5$ $X_5CX_8CX_5$ $X_5CX_9CX_4$ $X_4CX_{10}CX_4$
Pool 3	X_9 $X_2CX_2CX_2$ $X_2CX_3CX_2$ $X_2CX_4CX_2$
Pool 4	$X_2CX_5CX_2$ $X_2CX_6CX_2$ $X_2CX_7CX_2$
Pool 5	$X_2CX_8CX_2$ $X_2CX_9CX_2$ $X_2CX_{10}CX_2$
Pool 6	CCX_3CX_5C $CCX_5CX_4CX_4CC$
Pool 7	$CXCX_7CX_3CX_6$ $CX_6CX_6CCX_3CX_6C$

Selection Conditions - HER2-ECD was immobilized directly to maxisorp plates in 50 mM ammonium bicarbonate, pH 9.3, using 5 µg/ml, overnight at 4°C. Wells were blocked using PBS containing 1% BSA (PBS-BSA) for 1 hr at 25°C. The phage from the libraries described above were pooled into 7 groups as indicated in Table 1. Phage from each pool were incubated in wells containing HER2-ECD in PBS-BSA for 3 hr at 25°C; generally about 5×10^{10} phage were added at the beginning of each round. Unbound phage were removed by repetitive washing with PBS containing 0.05% Tween 20 (PBS-Tween); remaining phage were eluted with 500 mM KCl, 10 mM HCl, pH2. The eluted phage were then amplified in XL1-Blue cells with VCSM13 helper phage (Stratagene) overnight at 37 °C. IPTG (10 µM) was added only to the initial phage libraries and during amplification following round 1; later rounds relied on basal expression of the tac promoter for production of peptide-pVIII expression. Enrichment was monitored by titering the number of phage which bound to a target coated well compare to a well coated with BSA.

HER2 Phage Binding Assay- Inhibition of binding of phage displaying a single copy of peptide 1.1FI (Table 1) on their surface to the immobilized extracellular domain of Erb2 (HER2-ECD, Hudziak et al. (1991) J. Biol. Chem. 266:24109-15) was monitored using a phage ELISA. HER2-ECD was immobilized

directly to Maxisorp plates (Nunc) in 50 mM ammonium bicarbonate, pH 9.3, using

5 µg/ml, overnight at 4° C. Wells were blocked using PBS containing 1% BSA

(PBS-BSA) for 1 h at 25° C. Dilutions of 1.1FI-Fc in PBS-BSA were tested for

their ability to block the binding of 1.1FI displaying phage to the immobilized

5   HER2-ECD. The microtiter plate was washed with PBS containing 0.05% Tween20

(PBS-Tween) and the phage bound to HER2-ECD were detected with an anti-

gVIII/HRP monoclonal antibody conjugate (Amersham Pharmacia Biotech). The

amount of HRP bound was measured using ABTS/H_2O_2 substrate and monitoring the

change at 405 nm.

10   Results

Selection of Polyvalent Peptide-Phage that bind to HER2-ECD- Polyvalent

peptide libraries were sorted in 7 pools (**Table 1**)against immobilized HER2-ECD.

Polyvalent phage display (Scott et al. (1990) Science 249:386-390) was used to

enhance binding through avidity effects. Enrichment, the number of phage eluted

15   from a well coated with HER2-ECD divided by the number of phage of eluted from

a well coated with BSA, is shown in **Figure 4** for rounds 3 and 4. The DNA from

random clones in each pool were sequenced and the deduced peptide sequences are

presented in **Table** 2. Pool 1 was taken over by a single clone, while other

pools contained multiple sequences.

20   Using a phage binding assay, phage clones 1.1, 5.1 and 7.1 were found to

bind immobilized HER2-ECD, but not to immobilized BSA, or closely related HER3

or HER4 indicating they specifically recognized HER2-ECD (**Figure 5**). The

sequences of these clones were subjected to partial randomization in order to

re-select for higher affinity clones.

25   **Table 2**

clone	Sequence	SEQ ID NO:
Pool 1		
1.1	Y E V E A W D C M G P G C A N L F E A H	132
Pool 2		
2.1	S S E C A C D K G G R R V L C I N K V G	133
2.3	E P H G C S L W D W E L R T C S E Y A N	134
2.5	K E R P C A G D A P R K G V C H V A T H	135
2.6	K V R S C I E E S L D T R R C Y L V V E	136
2.7	A K T S S C G E H E E R R A V C V L S R	137
Pool 3		
3.1	K V W S V Q S P	138
3.2	G K V Q R C I P	139
3.3	Q T C R R V L C L P	140

3.4	R V W T W R W N	141
3.5	R I C T T P C A V	142
3.6	T S C R R V F C A V	143
3.7	R V C T G C V T	144
3.8	K V C T R V C C G T	145
Pool 4		
4.1	H P C H M R V L C A A	146
4.2	R G C K A T G K V L C S L	147
4.3	S G C L R A V G A C N T	148
4.4	A G C G S K A V C V S	149
4.5	R V W T A P Q C L I	150
4.6	K V C H A S S G C V A	151
4.7	R A C Q R A C L C P A	152
Pool 5		
5.1	R S C A D V A S R C W E H C I T	153
5.2	T D C G R V A S V C W E S C L I	154
Pool 6		
6.1	C C E T R W W C Q W G F C S G S A C C	155
6.2	G C K R V C S L G V M C	156
Pool 7		
7.1	C S W V L V Q C G G E W W H C C G L G C G L V V N A C	157
7.5	C G C E E R K A W K C Q E A C A R S G T V	158

EXAMPLE II

Methods

Partial or Complete Randomization on Monovalent Phage- Monovalent libraries which display a single copy of a peptide on the surface of phage fused through a linker sequence to the tail protein coded for by gIII were constructed using single-stranded template-directed mutagenesis (Kunkel et al. (1991) Met. Enz. 204:125-139) of a similar phagemid pB2479.g3. In this vector the coding sequence for gVIII has been replaced by a gIII, in addition, the CMP^r gene has been inserted into a unique hincII site in the AMP^r gene. The change in drug resistance was designed to eliminate contamination by related

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although weaker affinity polyvalent clones which could take over the population
through avidity effects (Cwirla et al. (1990) Proc. Natl. Acad. Sci. USA
87:6378-6382). Partially randomized libraries were designed to maintain a bias
towards the peptide sequences identified from the initial polyvalent libraries
5   while allowing a 50% mutation rate at each amino acid position. This mutation
rate was attained by synthesizing the oligos with a 70-10-10-10 mixture of
bases (where each base in the doped region of the oligo is coupled using a
mixture containing 70% of the base contributing to wildtype sequence and 10%
each of the other 3 bases). In contrast, complete randomization in libraries
10   was obtained by synthesizing oligos using NNS for particular codons in order
to fully randomize portions of a displayed peptide while keeping other portions
of the sequence constant.

Results

       Partial Randomization of Peptide-Phage - The initial peptide libraries
15   that were designed encoded a potential diversity of greater than 20^{20} (10^{26})
different clones while the actual libraries that were made contained
approximately only 10^9 clones; an incredibly small fraction of the potential
diversity. In order to narrow the search and yet further explore the peptide
diversity within the area of the initially selected peptides, a partial
20   randomization technique was employed. This technique maintains a bias towards
the wildtype sequence while introducing a 50 % mutation rate (at the amino acid
level) at each amino acid position; thus on average, a phage displayed 20 amino
acid peptide would acquire 10 random mutations. In addition, anticipation of
further affinity improvements led us to construct these libraries on monovalent
25   phage (gIII) (Bass et al., (1990) Proteins: Struct. Funct. Genet. 8:309; Lowman
et al., (1991) Biochemistry 30:10832) in order to eliminate avidity effects.

       Monovalent partial randomization libraries of clones 1.1, 5.1 and 7.1
were constructed and sorted for 4 rounds on HER2. Enrichment of >10,000 fold
was observed for libraries 1.1 and 7.1; however, no enrichment of library 5.1
30   was observed. Again, random clones were selected and sequenced; the deduced
peptide sequences from clones in libraries 1.1 and 7.1 are shown in Figure 15.
Several amino acid positions in libraries 1.1 and 7.1 were 100 % retained yet
multiple codons were observed at many of these positions. Residues strongly
retained following partial randomization may be crucial for binding either
35   through direct contacts or for structural reasons.

       Full maturation of HER2 binding sequences - To complete the affinity
maturation of the Class 1 sequences, a third set of libraries were constructed
which fixed positions that had been 100 % conserved and remaining positions
were fully randomize. In addition, the role of residues flanking the disulfide
40   loop of the Class 1 sequence was addressed by constructing libraries with
either portions of the amino and carboxy terminal missing. Thus, 3 monovalent
libraries were constructed as described in **Figure 16**. Enrichment of >500-fold
was observed by each library by round 4; the sequences from random clones are
presented in Figure 16. Even though these libraries which fully randomized up

to 10 positions were far from complete, a comparison of the full length and amino terminal truncated libraries demonstrated a preference for an additional disulfide in the C terminal region of the Class 1 sequence. In both libraries the additional disulfide is found in 2 locations. In the third library,

5   truncation of the C terminal positions preclude the selection of an additional disulfide at these locations. Aside from the selection of an additional disulfide, the residues selected at other randomized positions are quite diverse.

EXAMPLE III

10   Methods

   Peptide Synthesis- Peptides were synthesized by either manual or automated (Milligen 9050) Fmoc-based solid phase synthesis on a 0.25 mmol scale using a PEG-polystyrene resin (Bodanszky et al. (1984) Int. J Peptide and Protein Res. 23(1):111) . Side chain protecting groups were removed and the

15   peptide was cleaved from the resin with 95 % trifluoroacetic acid (TFA) and 5 % triisopropylsilane. A saturated iodine solution in acetic acid was added to oxidation of disulfide bonds. Peptides were purified by reversed-phase HPLC using a water/acetonitrile gradient containing 0.1 % TFA. Peptides were >95 % pure by analytical HPLC and its identity verified by mass spectrometry.

20   *Production of peptide-Z fusions* - Phage peptides selected for binding to HER2-ECD were expressed and secreted from *E. coli* (27C7) as fusions to the Z domain from protein A. Oligos were designed to insert the coding sequence for the phage-peptide sequences between the stII signal sequence and the Z domain in a plasmid containing the Z domain (pZCT). Cells were grown in phosphate

25   limiting media and peptide-Z fusions were purified from the media using an IgG affinity column as described (Dennis et al., (1993) Proteins: Struct. Funct. Genentics 15:312-321). IgG affinity purified peptide Z-fusion was biotinylated using NHS-LC-biotin (Pierce) and characterized by mass spectrometry.

   Peptide-Z fusions which formed multimers could be separated into monomer

30   and dimer containing fractions by size exclusion chromatography on a Superdex 75 column in PBS.

   Removal of the Z domain from purified peptide-Z fusions - Removal of the Z domain from purified peptide-Z fusions was accomplished by digestion of the fusion with trypsin. Trypsin (1 %, w/w) was added for 4 hrs at 37°C in 100 mM

35   Tris pH 8.0 and 10 mM CaCl$_2$. The peptide, free of the Z domain was then purified on a C18 reverse-phase HPLC column using a 20 to 50 % acetonitrile gradient in 0.1 % TFA.

   Construction of Her2-Fc expression vector—Standard recombinant DNA techniques were used for the construction of a recombinant transfer vector

40   based on the vector pVL1393 (Pharmigen) (Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, second Ed., Cold Spring Harbor Laboratory Press, New York; O'Reilly, D. R., Miller, L. K., and Luckow, V. A. (1994) *Baculovirus Expression Vectors: A Laboratory Manual*,

Oxford University Press, New York). The pVL1393 derived plasmid pbPH.His was linearized with *Nco* I and *Sma* I and treated with shrimp alkaline phosphatase (Dwyer, M. A. et al. (1999) J. Biol. Chem. 274:9738-9743). The Fc portion of the human IgG1 was obtained as a 700 base pair fragment by restriction
5   digestion using *Nde* I and subsequent treatment with Klenow and *Nco* I of another pVL1393 derived plasmid pVL1393.IgG. The signal sequence for MIP.5 was introduced before the Fc sequence as a PCR fragment digested with *EcoR* I, included within the fragment is an *Asc* I site. The *Asc* I site occurs following the putative signal sequence cleavage site. Following ligation, competent *E.*
10 *coli* XL-1 Blue were transformed and bacteria were selected for the correct recombinant plasmid (pVL1393.MIP.5sig.Fc) by DNA sequence analysis. Then, pVL1393.MIP.5sig.Fc was linearized with *Asc* I and *Stu* I and treated with shrimp alkaline phosphatase. The linearized vector was then ligated with a synthetic piece of DNA with compatible ends. The synthetic DNA insert was formed by
15 annealing 2 oligos with the sequences: 5'-CGC GCC CAG GTG TAC GAG TCC TGG GGA TGC ATC GGC CCC GGC TGC GCC TGC CTG CAG GCC TGC CTG GGA GGC GGG AGC TCC GGC-3' (SEQ ID NO:159) and 5'-GCC GGA GCT CCC GCC TCC CAG GCA GGC CTG CAG GCA GGC GCA GCC GGG GCC GAT GCA TCC CCA GGA CTC GTA CAC CTG GG-3' (SEQ ID NO:160) coding for peptide sequence 1.1FI including a GGGSSG (SEQ ID NO:130) linker.
20   Following ligation, competent *E. coli* XL-1 Blue were transformed and bacteria were selected for the correct recombinant plasmid (termed pVL.1393.MIP5.1.1FI-Fc) by DNA sequence analysis using the dRhodamine dye-terminator method and an Applied Biosystems ABI Model 373 automated DNA sequencer. Recombinant transfer vector was purified using a Qiagen Mini-Prep
25 and used for construction of recombinant baculovirus.
        Recombinant baculovirus was generated following cotransfection of Sf9 cells with the transfer vector and the linearized wild type baculovirus DNA (*Autographa californica* nuclear polyhedrosis virus (AcNpV), Pharmingen). A primary amplification of the recombinant baculovirus achieved detectable
30 protein expression. Subsequent plaque-purification and titering of the viral stock was performed by plaque assays. Standard methods were utilized as previously described (O'Reilly, D. R., Miller, L. K., and Luckow, V. A. (1994) *Baculovirus Expression Vectors: A Laboratory Manual*, Oxford University Press, New York).
35   *Cell Culture*—Adherent cultures of *Spodeptera frugiperda* (Sf9) insect cells (ATCC CRL 1711) were maintained at 28 °C in Hink's TNM-FH insect medium supplemented GRACE's (JRH Biosciences, #51942-78P), with glutamine, streptomycin/penicillin, and 10% fetal bovine serum (heat inactivated for 30 min at 56 °C). Cultures were passaged every 3 days. Spinner cultures of High
40 5 cells (Trichoplusia ni, BT1.TN.SB1-4 (Invitrogen)) (500 ml at 2.0×10^6 cells/ml) were infected at a multiplicity of infection of 0.5 and harvested 60 h posttransfection. Suspension cultures were maintained in spinner flasks at 28 °C using ESF-921 protein free insect cell culture medium (Expression Systems

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LLC, #96-001). Cultures were passaged every 3 days to a starting cell density of 10^6 cells/ml.

   Peptide-Fc Purification—Following the optimized infection protocol, the High 5 cells were removed by centrifugation at 800 x g at 4 °C for 10 min. The

5 clarified supernatant (0.5 L) was filtered using a 0.45 µ Nalgene filter and applied to a 0.5 ml Hi-Trap Protein A Column (Amersham Pharmacia Biotech) equilibrated with PBS (phosphate buffered saline) at 25 °C. After washing with 20 ml of PBS, the column was eluted with 3 ml of 0.2 N HOAc and fractions containing peptide-Fc were lyophilized and stored at 4 °C.

10   *SDS-PAGE* —Samples were analyzed reduced and unreduced on a 4-20% Tris-glycine SDS-PAGE (Novex) along with prestained protein molecular weight markers (SeaBlue, Novex) using the method of Laemmli (Laemmli, U. K. (1970) Nature 227:680-685).

   Protein Sequencing— 1.1FI-Fc purified from the infected Sf9 cell

15 supernatants was subjected to SDS-PAGE, and then transferred to a PVDF membrane. Electroblotting onto Millipore Immobilon-PSQ membranes was carried out for 1 h at 250 mA constant current in a BioRad Trans-Blot transfer cell (Matsudaira, P. (1987) J. Biol. Chem. 262: 10035-10038). The PVDF membrane was stained with 0.1% Coomassie Blue R-250 in 50% methanol, 0.5 min and destained

20 for 2-3 min with 10% acetic acid in 50% methanol. The membrane was thoroughly washed with water and allowed to dry before storage at -20 °C. The 1.1FI-Fc band at about 50 kD was cut out and the first 11 residues were sequenced using a model 494A Applied Biosystems sequencer equipped with an on-line PTH analyzer. Peaks were integrated with Justice Innovation software using Nelson

25 Analytical 760 interfaces. Sequence interpretation was performed on a DEC alpha (Henzel, W. J., Rodriguez, H., and Watanabe, C. (1987) J. Chromatog. 404: 41-52).

   HER2 Competition ELISA- 1.1FI-Fc binding to HER2-ECD was monitored using a competition ELISA. Samples were titered in PBS-BSA and tested for their

30 ability to block the binding of 40 nM biotinylated 1.1.FI-Z to HER2-ECD immobilized on microtiter plates as described above. Following a 1 h incubation the plate was washed with PBS-Tween and Strepavidin/HRP conjugate (Streptavidin-POD, Roche Molecular Biochemicals) was added for 30 min. Plates were washed again with PBS-Tween and the bound HRP was assayed using ABTS/H_2O_2

35 substrate (Kirkegaard & Perry Laboratories) and the absorbance at 405 nm was monitored. The absorbance at 405 nm was plotted versus the concentration of 1.1FI-Fc originally added to the well. Sigmoidal curves were fit to a four parameter equation by nonlinear regression analysis (Marquardt, J. Soc. Indust. Appl. Math. 11:431-441 (1963); the concentration of 1.1FI-Fc required to give

40 a half-maximal signal in the assay was calculated from the curves and is referred to as the IC_{50} value.

   Cell binding- Cells expressing HER2 (BT 474 [3+], MDA 361 [2+], and BaF3 [0+]) were detached with trypsin, washed twice with Wash Buffer (PBS containing 0.5% BSA, 1 mM NaOH), and split into fractions containing 0.5 x 10^6 cells each. The

cells were suspended in Wash Buffer containing 0, 0.5 or 5 µM 1.1.FI-Zb, washed twice with Wash Buffer and suspended in Wash Buffer containing strepavidin-PE, washed twice with Wash Buffer and subjected to FACS analysis.

Results

5   *Characterization of Peptides that bind to HER2-ECD* - Peptide HER201 was synthesized corresponding to partially randomized phage-clone 1.1.2 (Figure 15). In addition, peptide HER212 corresponding to a representative clone 1.1.FI (Figure 16) derived from the fully randomized full length library was also synthesized. Due to the presence of 2 disulfides in this peptide,
10   orthogonal protecting groups were used for the cysteines in order to induce a 1-2 and 3-4 disulfide arrangement. This arrangement was suspected because of the order in which the disulfide bonds evolved during the selection process.

       Additionally, this peptide and a peptide sequence derived from the consensus of library 7.1 (Figure 15) (7.1c) were expressed and secreted from
15   *E. coli* as a fusion to the Z domain of protein A (called 1.1.FI-Z and 7.1c-Z) where cysteine oxidation might be allowed to occur as it does during phage production. The 1.1.FI-Z preparation was biotinylated (1.1.FI-Zb) and bound to immobilized HER2 with an EC50 of 2 nM based on the total protein concentration (**Figure 6**) and served as a reagent for the competition ELISA.

20   Peptides HER201, HER212 as well as the recombinant peptide fusions 1.1.FI-Z and 7.1c-Z were tested for the ability to block binding of 1.1.FI-Zb to immobilized HER2 (**Figure 7**). HER201 and 7.1c-Z had IC50s of 300 and 1,100 nM respectively. Interestingly, HER212 and 1.1.FI-Z, which were derived from the same phage-peptide sequence had dramatically different IC50s: 9 µM and 50
25   nM respectively. One explanation could be that another disulfide arrangement was forced in the synthesis of HER212. Additionally, SDS-PAGE analysis of 1.1.FI-Z (**Figure 8B**)(lane S) revealed a heterogeneous mixture likely due to multimerization caused by intermolecular disulfides. Mass spectral analysis confirmed presence of dimer 1.1.FI-Z while HER212 was present only as monomer.

30   The 1.1.FI-Z preparation was further purified using a Superdex 75 into monomer and dimer containing fractions (**Figure 8A and 8B**). Surprisingly, HER2 binding activity coincided with the dimer fractions rather than the monomer fractions (**Figure 8B and 8C**) when assayed in the HER2 competition ELISA.

       In addition, 1.1.FI-Z was digested with trypsin to removed the Z domain
35   and subjected to reversed phase HPLC. Fractions from the HPLC were tested for their ability to compete in the HER2 competition ELISA. The active fraction had a mass corresponding to the sequence of HER212 but was also found to contain dimer. This fraction had an IC50 of 3 nM in the HER2 competition ELISA based on protein concentration.

40   In an attempt to facilitate dimer formation, an expression vector was constructed in which the amino acid sequence of 1.1.FI was repeated twice prior to fusion with Z domain (**Figure 9**). While this "single chain dimer" (called (FI)$_2$-Z), also produced dimer when expressed, the dominate species formed was

monomer (**Figure 9B**). In addition, when fractionated on a Superdex 75 column (**Figure 9A**) and assayed in the HER2 competition ELISA, the active species was now identified as monomer (**Figure 9B and C**). (FI) $_2$-Z has a similar affinity for HER2 as the 1.1.FI-Z dimer (IC50 = 20 nM).

5   *Binding Site on HER2* - To test whether Class 1 and Class 7 sequences bound to the same or independent sites on HER2, the binding of phage clones from Class 1 and Class 7 libraries to immobilized HER2 was examined in the presence of HER201 or 7.1c-Z. From **Figure 10A and 10B**, it is apparent that HER201 and 7.1c-Z block the binding of both Class 1 and Class 7 phage

10   suggesting that both Classes bind to the same site on HER2. A closer look at the sequences obtained in these 2 classes reveals a core homology region (**Figure 11**).

         The binding site of the Class 1 and Class 7 peptides also appears to be a novel site on HER2 in that known monoclonal antibodies to HER2 did not

15   directly compete with 1.1.FI-phage binding to immobilized HER2 (**Figure 12**). Increasing concentrations of various monoclonal antibodies were added to block 1.1.FI-phage binding to immobilized HER2 without effect. The reduction in binding at very high concentrations of 3H4 (>100 nM) are thought to result through an indirect effect since the affinity of this monoclonal antibody for

20   HER2 is below 100 pM.

         Cell Binding - 1.1.FI-Zb was tested for its ability to recognize HER2 expressed on cells. Three cell types which expressed HER2 at different levels were examined: BT 474 [3+] (3×10^6 receptors/cell), MDA 361 [2+] (4×10^5 receptors/cell), and a control cell line that doesn't express HER2, BaF3. By

25   FACS analysis, 1.1.FI-Zb was found to bind to cells proportional to the number of HER2 of receptors expressed/cell and the concentration of 1.1.FI-Zb used (**Figure 13**).

         Peptide-Fc fusions - DNA coding for the 1.1.FI sequence was fused through a linker to an Fc from a IgG$_1$ and expressed in bacolovirus. The resulting

30   protein was purified on a Protein A column and characterized under oxidizing and reducing conditions by SDS-PAGE (**Figure 14**). A clear molecular weight shift is observed under these 2 conditions suggesting dimerization of the Fc. This molecule, when tested for its ability to block 1.1FI phage binding to immobilized HER2, has an IC50 of 3 nM. When tested in the HER2 competition

35   ELEISA, 1.1FI-Fc had a similar IC50 to 1.1FI-Z indicating that the 1.1FI sequence was fully capable of binding HER2 when fused to an Fc (Figure 17).

WHAT IS CLAIMED IS:

1.   A non-naturally occurring peptide ligand which competes for binding HER2
in an _in vitro_ assay with a peptide ligand having the formula:

$Xaa_{(1-14)}$-Cys-Xaa_{16}-Gly-Pro-Gly-Cys-$Xaa_{(21-27)}$ (SEQ ID NO:90)

wherein $X_{(1-14)}$ is absent or between one and fourteen amino acids; Xaa16 is an
amino acid selected from the group consisting of Met, Thr, Cys and Ile and
Xaa(21-27) is absent or between one and 7 amino acids.

2.   The peptide ligand of claim 1 having the following formula:

$Xaa_{(1-7)}$-Cys-Xaa_9-Gly-Pro-Gly-Cys-$Xaa_{(14-20)}$ (SEQ ID NO:92)

wherein $X_{(1-7)}$ is absent or between one and seven amino acids; and Xaa_9 is an
amino acid selected from the group consisting of Met, Ile, Thr or Cys and
$Xaa_{(14-20)}$ is absent or between one and seven amino acids.

3.   The peptide ligand of claim 2 having the following formula:

$Xaa_{(1-7)}$-Cys-Ile-Gly-Pro-Gly-Cys-$Xaa_{(14-20)}$ (SEQ ID NO:161).

4.   A peptide ligand having the formula A-A wherein A is the peptide ligand
of claim 3 and "-" is an optional linker domain.

5.   The peptide ligand of claim 3 having the following formula:

$Xaa_{(1-5)}$-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-$Xaa_{(14-20)}$ (SEQ ID NO:93)

wherein $Xaa_{(1-5)}$ is absent or between one and five amino acids.

6.   The peptide ligand of claim 5 having the following formula:

$Xaa_{(1-3)}$-Glu-Xaa_5-Trp-Gly-Cys-Ile-Gly-Pro-Gly-Cys-Xaa_{14}-Xaa_{15}-Leu-$Xaa_{(17-20)}$ (SEQ ID NO:87)

wherein $Xaa_{(1-3)}$ is absent or between one and three amino acids, Xaa_5 is an
amino acid,

Xaa_5 is an amino acid,

Xaa_{14} is an amino acid,

Xaa_{19} is an amino acid and

-$Xaa_{(17-20)}$ is absent or between one and four amino acids.

7.   The peptide ligand of claim 6 wherein $X_{(1-3)}$- is absent or selected from
the group consisting of

Gln-Arg-Asn-

Leu-Ser-Pro-

Glu-Asn-Trp-

Ala-Ser-His-

Lys-Leu-Asn-

Thr-Gln-Ala-

Ala-Pro-Arg-

```
      Gln-Val-Tyr-
      Arg-Thr-Glu-
      Phe-Ala-Gly-
      Thr-Ala-Arg-
 5    Arg-Pro-His-
      Asn-Val-Cys-
      Cys-Ile-Asp-
      Tyr-Glu-Trp-
      Arg-Trp-Asp-
10    His-Trp-Met-
      Asn-Trp-Pro-
      Phe-Asn-Trp-
      Phe-Ser-Gly-
      Gly-Gly-Trp-
15    Leu-Trp-Phe-
      Gly-Ile-Pro-
      Trp-Trp-Thr-
      Leu-Gly-Trp-
      Ser-Pro-Trp-
20    Arg-Gly-Trp-,
```

Xaa_5 is selected from the group consisting of:

Ala, Thr, Met, Val, Arg, Glu, Asp, Ser, Gln, Pro, Gly, Phe and Lys,

Xaa_{14} is selected from the group consisting of:

Glu, Lys, Arg, Asp, Ser, Ala, Asn, Gly, Pro and Gln,

25   Xaa_{15} is selected from the group consisting of:

Met, Phe, Ala, Met, Cys, Gln, Glu, Ala, Trp, Phe, Leu, Val, Tyr, Trp, and

$-Xaa_{(17-20)}$ is a four amino acid peptide having the following formula:

$-Xaa_{17}-Xaa_{18}-Cys-Xaa_{20}$.

8.   The peptide ligand of claim 7 wherein $-Xaa_{(17-20)}$ is selected from the

30   group consisting of:

```
      -Gln-Ala-Cys-Met  (SEQ ID NO:102)
      -Leu-Gln-Cys-Trp  (SEQ ID NO:103)
      -Met-Ser-Cys-Val  (SEQ ID NO:104)
      -Leu-Arg-Cys-Ile  (SEQ ID NO:105)
35    -Gln-Ala-Cys-Leu  (SEQ ID NO:106)
      -Leu-Ser-Cys-Leu  (SEQ ID NO:107)
      -Ile-Gly-Cys-Leu  (SEQ ID NO:108)
      -Leu-Ala-Cys-Leu  (SEQ ID NO:109)
      -Leu-Ser-Cys-Ile  (SEQ ID NO:110)
40    -Met-Asn-Cys-Leu  (SEQ ID NO:111)
      -Leu-Arg-Cys-Leu  (SEQ ID NO:112)
      -Leu-Lys-Cys-Leu  (SEQ ID NO:113)
      -Leu-Gly-Cys-Leu  (SEQ ID NO:114)
```

-Leu-Asn-Cys-Ile (SEQ ID NO:115)

-Met-Gly-Cys-Leu (SEQ ID NO:116) and

-Met-Ala-Cys-Leu (SEQ ID NO:117).

9.   The peptide ligand of claim 6 wherein $-Xaa_{(17-20)}$ is a four amino acid
5   peptide having the following formula
            $-Cys-Xaa_{18}-Xaa_{19}-Cys$
wherein Xaa_{18} is an amino acid and Xaa_{19} is an amino acid.

10.   The peptide ligand of claim 9 wherein $-Xaa_{(17-20)}$ is selected from the
group consisting of:
10   -Cys-Ala-Trp-Cys (SEQ ID NO:118)

-Cys-Ser-Trp-Cys (SEQ ID NO:119)

-Cys-Glu-Pro-Cys (SEQ ID NO:120)

-Cys-Asp-Trp-Cys (SEQ ID NO:121)

-Cys-Glu-Trp-Cys (SEQ ID NO:122)

15   -Cys-Asn-Trp-Cys (SEQ ID NO:123) and

-Cys-Gly-Trp-Cys (SEQ ID NO:124).

11.   The peptide ligand of claim 1 having the formula:

$Xaa_{(1-10)}-Glu-Xaa_{12}-Trp-Xaa_{14}-Cys-Cys-Gly-Pro-Gly-Cys-Xaa_{21}-Xaa_{22}-Xaa_{23}-Xaa_{(24-27)}$

(SEQ ID NO:126)

20   wherein $Xaa_{(1-10)}$ is absent or between 1 and 10 amino acids;

Xaa_{12} is an amino acid;

Xaa_{14} is an amino acid;

Xaa_{21} is an amino acid;

Xaa_{22} is an amino acid;

25   Xaa_{23} is an amino acid selected from the group consisting of Val and Leu and

$Xaa_{(24-27)}$ is absent or between one and four amino acids.

12.   The peptide ligand of claim 11 wherein $Xaa_{(1-10)}$ is a 10 amino acid
peptide having the following formula:

$-Cys-Xaa_{(2-7)}-Cys-Xaa_{9}-Gly-;$ $Xaa_{(24-27)}$ is a four amino acid peptide having the
30   following formula:

$Xaa_{(24-26)}-Cys$

wherein $Xaa_{(2-7)}$ is a six amino acid peptide and

$Xaa_{(24-26)}$ is a 3 amino acid peptide.

13.   A peptide ligand having the formula B-B wherein B is the peptide ligand
35   of claim 12 and wherein "-" is an optional linker domain.

14.   A peptide ligand having the formula A-B wherein A is the peptide ligand
of claim 2 and B is the peptide ligand of claim 11 and wherein "-" is an

-52-

optional linker domain.

15.   The peptide ligand of claim 12 wherein Xaa$_{(1-10)}$ has the following formula:

Cys-Xaa$_2$-Trp-Val-Xaa$_5$-Xaa$_6$-Xaa$_7$-Cys-Xaa$_9$-Gly- (SEQ ID NO:127).

5   16.   The peptide ligand of claim 15 wherein Xaa$_{(1-10)}$ has the following formula:

Cys-Xaa$_2$-Trp-Val-Xaa$_5$-Xaa$_6$-Xaa$_7$-Cys-Xaa$_9$-Gly- (SEQ ID NO:127).

and Xaa$_2$ is an amino acid selected from the group consisting of Ala and Ser;

Xaa$_5$ is an amino acid selected from the group consisting of Ser, Leu, Ala, Arg

10   and Val;

Xaa$_6$ is an amino acid selected from the group consisting of Phe, Val and Leu;

Xaa$_7$ is an amino acid selected from the group consisting of Asp Gln, Tyr, Trp,

Leu and His and

Xaa$_9$ is an amino acid selected from the group consisting of Gly, Phe and Leu.

15   17.   The peptide ligand of claim 16 wherein Xaa$_{(1-10)}$ has the following formula:

Cys-Ser-Trp-Val-Leu-Xaa$_6$-Xaa$_7$-Cys-Gly-Gly- (SEQ ID NO:162).

18.   The peptide ligand of claim 17 wherein Xaa$_{(24-26)}$ has the following formula:

20   Xaa$_{24}$-Xaa$_{25}$-Xaa$_{26}$ and

Xaa$_{24}$ is an amino acid selected from the group consisting of Trp, Val, Gly and

Ala;

Xaa$_{25}$ is an amino acid selected from the group consisting of Asn, Lys, Asp, Glu

and His and

25   Xaa$_{26}$ is an amino acid selected from the group consisting of Ala, Ser and Val.

19.   The peptide ligand of claim 17 wherein Xaa$_{(24-26)}$ has the following formula:

Xaa$_{24}$-Xaa$_{25}$-Xaa$_{26}$ and

Xaa$_{24}$ is an amino acid selected from the group consisting of Trp, Val, Gly and

30   Ala;

Xaa$_{25}$ is an amino acid selected from the group consisting of Asn, Lys, Asp, Glu

and His and

Xaa$_{26}$ is an amino acid selected from the group consisting of Ala, Ser and Val.

20.   The peptide ligand of claim 19 wherein

35   Xaa$_{24}$ is Val;

Xaa$_{25}$ is Asn and

Xaa$_{26}$ is Ala.

21.   A polypeptide which comprises:

(a)   a peptide ligand according to any of claims 1, 4, 11, or 13 and

(b)   an immunoglobulin constant region sequence.

22.   The polypeptide of claim 21 further comprising a linker sequence.

23.   The polypeptide of claim 20 or 21 wherein the immunoglobulin constant region sequence is the constant domain of an IgG heavy chain.

24.   The polypeptide of claim 22 wherein said constant region sequence comprises the CH3 domain of an immunoglobulin heavy chain.

25.   The polypeptide of claim 24 further comprising the hinge region of an immunoglobulin heavy chain.

26.   The polypeptide of claim 25 wherein said constant region sequence comprises at least functionally active hinge, CH2 and CH3 domains of the constant region of an immunoglobulin heavy chain.

27.   The polypeptide of claim 21 further comprising an additional functional moiety.

28.   The polypeptide of claim 27 wherein the additional functional moeity is selected from the group consisting of a cytotoxic agent and an enzyme.

29.   The polypeptide of claim 27 wherein the additional functional moiety is a cytotoxic agent.

30.   A pharmaceutical composition comprising the peptide ligand of claim 1 and pharmaceutically acceptable excipient.

31.   A pharmaceutical composition comprising the polypeptide of claim 21 and a pharmaceutically acceptable excipient.

32.   A pharmaceutical composition comprising the polypeptide of claim 28 and a pharmaceutically acceptable excipient.

33.   An isolated DNA molecule encoding the peptide ligand of claim 1.

34.   The DNA molecule of claim 33 further comprising an expression control sequence operably linked to the DNA molecule.

35. An expression vector comprising the DNA molecule of claim 33 wherein the control sequence is recognized by a host cell transformed with the vector.

36. A host cell transformed with the vector of claim 35.

37. A method for expressing a DNA molecule encoding a peptide ligand in a host cell, comprising culturing the host cell of claim 36 under conditions suitable for expression of the peptide ligand.

38. The method of claim 37 further comprising recovering the peptide ligand from the culture medium.

Fig.1

```
             230        240        250        260        270
humIgG1  PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
humIgG2  PAP-PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV
humIgG3  PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYV
humIgG4  PAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYV
murIgG1  ---TVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQFSWFV
murIgG2A PAPNLLGGPSVFIFPPNIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFV
murIgG2B PAPNLEGGPSVFIFPPNIKDVLMISLTPKVTCVVVDVSEDDPDVQISWFV
murIgG3  PPGNILGGPSVFIFPPKPKDALMISLTPKVTCVVVDVSEDDPDVHVSWFV
```

```
             280        290        300        310        320
humIgG1  DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
humIgG2  DGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLP
humIgG3  DGVEVHNAKTKPREEQFNSTFRVVSVLTVLHQDWLNGKEYKCKVSNKALP
humIgG4  DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLP
murIgG1  DDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDCLNGKEFKCRVNSAAFP
murIgG2A NNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLP
murIgG2B NNVEVHTAQTQTHREDYNSTIRVVSHLPIQHQDWMSGKEFKCKVNNKDLP
murIgG3  DNKEVHTAWTQPREAQYNSTFRVVSALPIQHQDWMRGKEFKCKVNNKALP
```

```
             330        340        350        360        370
humIgG1  APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
                        D    L
humIgG2  APIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
humIgG3  APIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
humIgG4  SSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV
murIgG1  APIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITV
murIgG2A APIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYV
murIgG2B SPIERTISKPKGLVRAPQVYTLPPPAEQLSRKDVSLTCLVVGFNPGDISV
murIgG3  APIERTISKPKGRAQTPQVYTIPPPREQMSKKKVSLTCLVTNFFSEAISV
```

```
             380        390        400        410        420
humIgG1  EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
humIgG2  EWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
humIgG3  EWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMH
humIgG4  EWZSNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVLH
murIgG1  EWQWNGQPAENYKNTQPIMDTDGSYFVYSKLNVQKSNWEAGNTFTCSVLH
murIgG2A EWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVH
murIgG2B EWTSNGHTEENYKDTAPVLDSDGSYFIYSKLNMKTSKWEKTDSFSCNVRH
murIgG3  EWERNGELEQDYKNTPPILDSDGTYFLYSKLTVDTDSWLQGEIFTCSVVH
```

```
             430        440
humIgG1  EALHNHYTQKSLSLSPGK
humIgG2  EALHNHYTQKSLSLSPGK
humIgG3  EALHNRFTQKSLSLSPGK
humIgG4  EALHNHYTQKSLSLSLGK
murIgG1  EGLHNHHTEKSLSHSPGK
murIgG2A EGLHNHHTTKSFSRTPGK
murIgG2B EGLKNYYLKKTISRSPGK
murIgG3  EALHNHHTQKNLSRSPGK
```

Fig. 2A

Percent Identity Among Fc Sequences

	1	2	3	4	5	6	7	8
1. humIgG1	-	94	94	94	64	66	63	68
2. humIgG2		-	93	92	65	63	60	67
3. humIgG3			-	91	64	64	61	67
4. humIgG4				-	62	64	61	64
5. murIgG1					-	65	61	67
6. murIgG2A						-	77	70
7. murIgG2B							-	68
8. murIgG3								-

Fig. 2B

```
            230       240       250       260       270
humIgG1     PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
humIgG2     PAP-PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV
humIgG3     PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYV
humIgG4     PAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYV
                   * * * *                              *       *  *

            280       290       300       310       320
humIgG1     DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
humIgG2     DGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLP
humIgG3     DGVEVHNAKTKPREEQFNSTFRVVSVLTVLHQDWLNGKEYKCKVSNKALP
humIgG4     DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLP
                           *       *          *                  *

            330       340       350       360       370
humIgG1     APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
                                 D L
humIgG2     APIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
humIgG3     APIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
humIgG4     SSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV
            * *            *                 *

            380       390       400       410       420
humIgG1     EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
humIgG2     EWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
humIgG3     EWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMH
humIgG4     EWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMH
                  *          *         *              *        *  *

            430       440
humIgG1     EALHNHYTQKSLSLSPGK
humIgG2     EALHNHYTQKSLSLSPGK
humIgG3     EALHNRFTQKSLSLSPGK
humIgG4     EALHNHYTQKSLSLSLGK
                 * *              *
```

Fig. 3

Figure 4

Figure 5

Binding Specificity of Polyvalent Phage Clones
Selected from Naive Libraries

Figure 6.

EC_{50} for 1.1.Fl-Zb Binding to Immobilized HER2

Figure χ

HER2 Competition ELISA

Legend:
- HER201 (2 Cys)
- HER212 (4C$_{syn}$)
- 1.1.Fl-Z (4 Cys$_{recomb.}$)
- 7.1c-Z (6C)
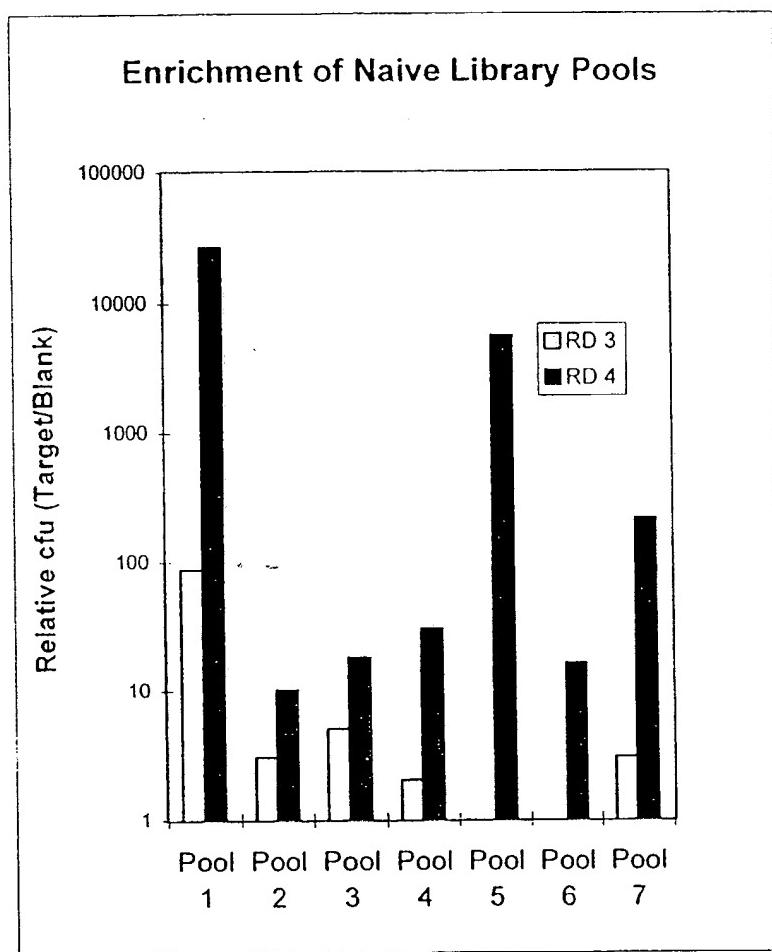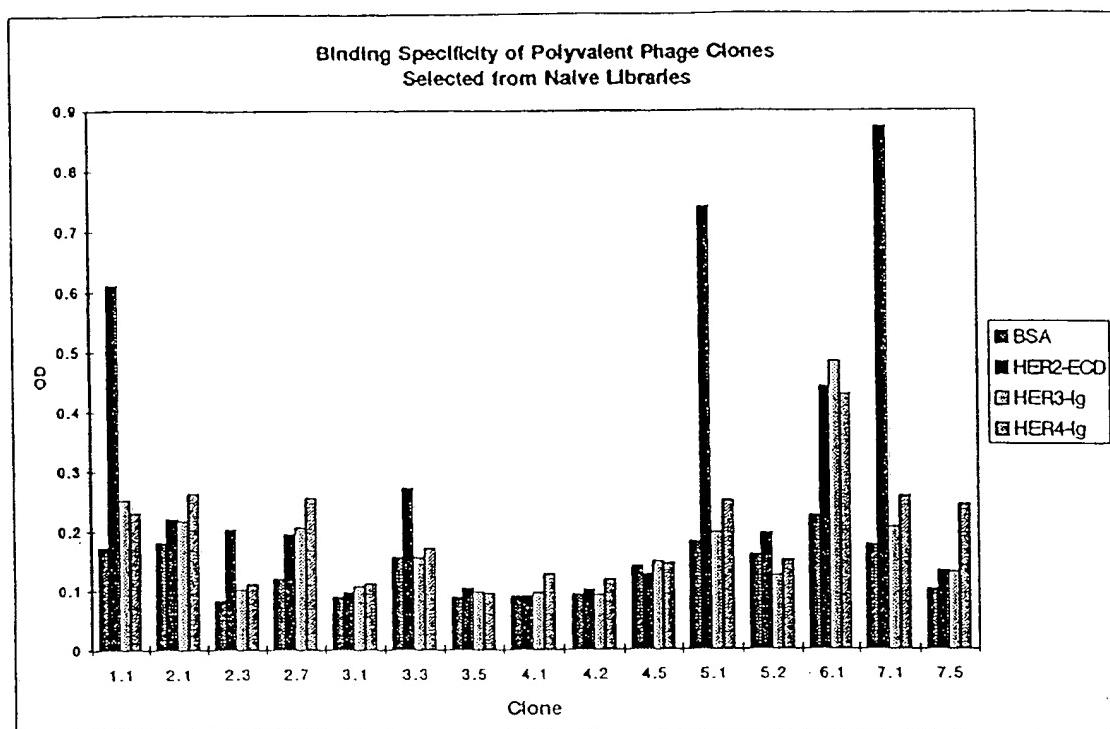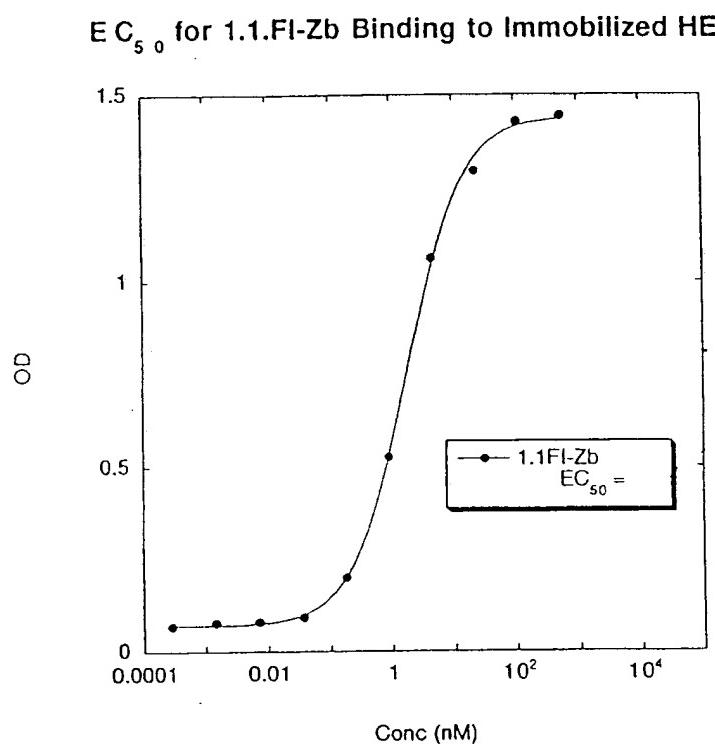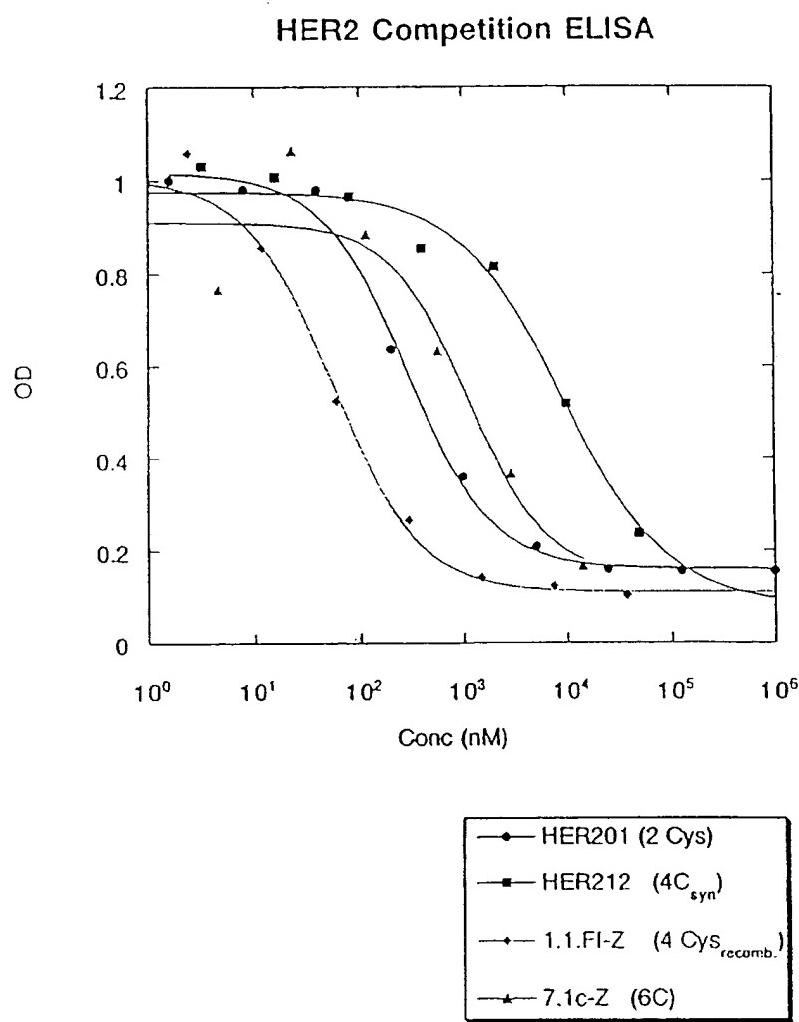
Figure 4

1.1.FI-Z

QVYESWGCIGPGCACLQACL-Z

A.

S

B.

97 —
66 —
45 —
31 —
21 —
14 —

←— dimer
←— monomer

S   D   M

C.

OD

1.4
1.2
1
0.8
0.6
0.4
0.2
0

M

S

D

10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2}

Conc (µM)
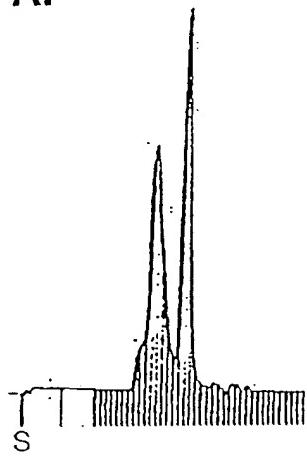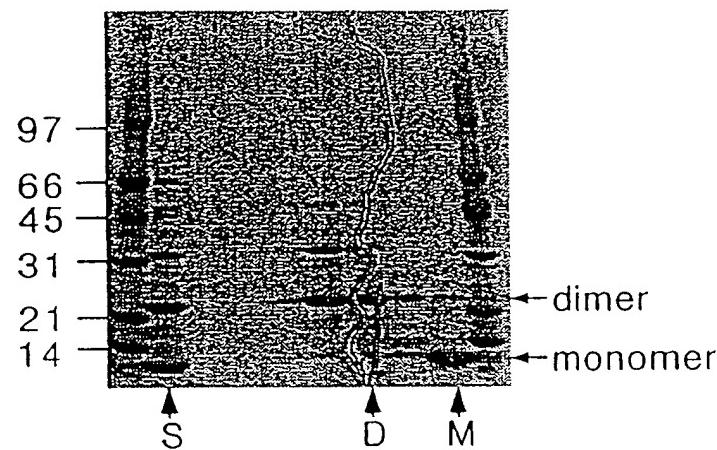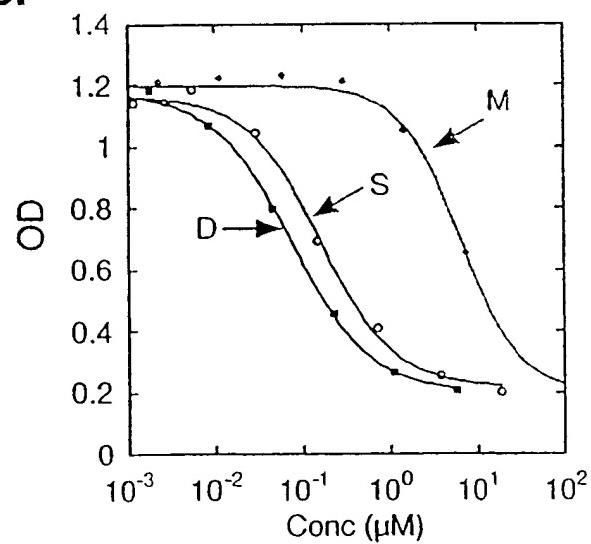
Figure 9

(1.1.Fl)₂-Z

QVYESWGCIGPGCACLQACLGGGSGGQVYESWGCIGPGCACLQACL-Z

A.

B.

C.

Figure (0

Class 1 and 7 Phage binding to HER2 is blocked by HER201 peptide

Class 1 and 7 Phage binding to HER2 is blocked by Peptide 7.1c-Z
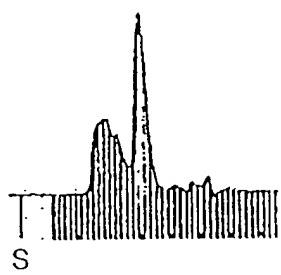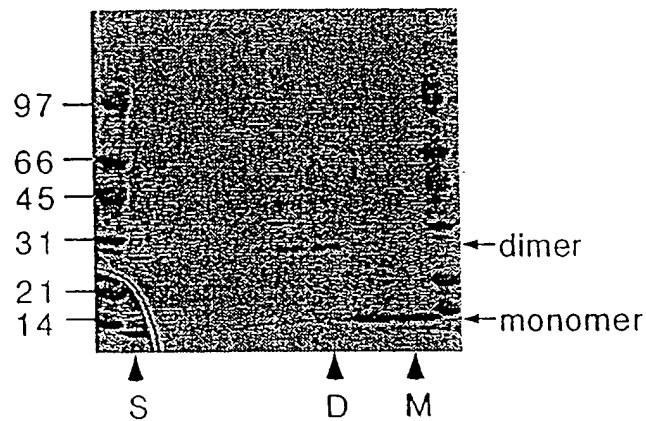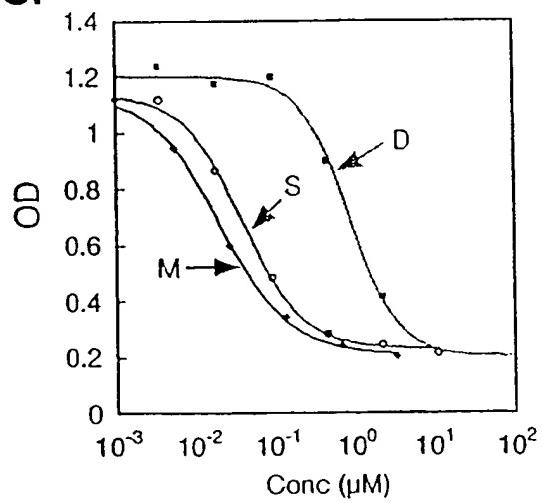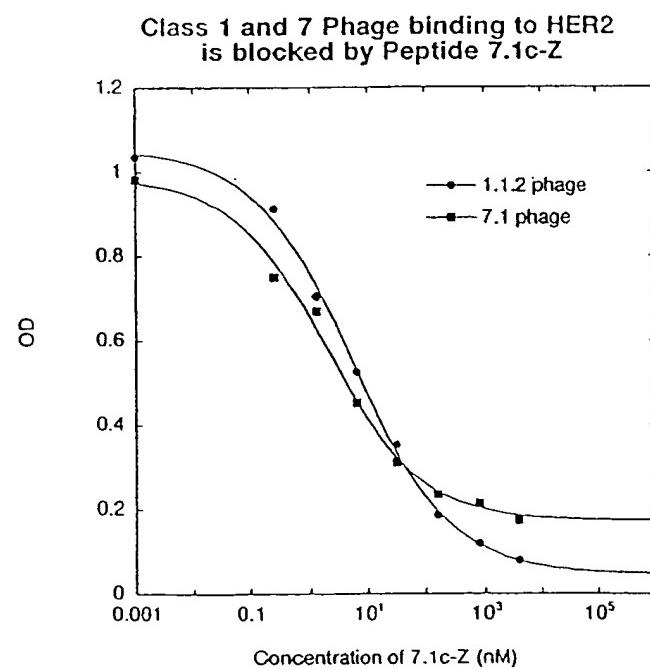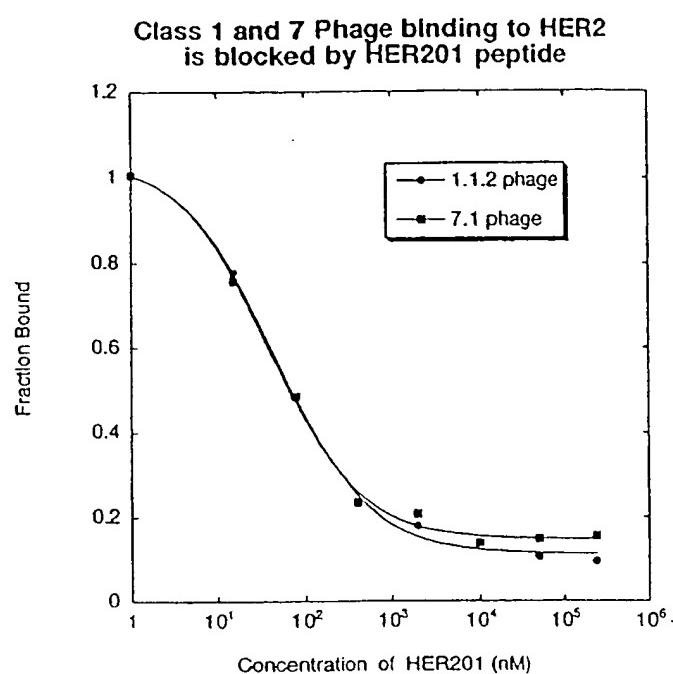
Figure 11

Class 7

C	A	W	V	S	V	E	C	G	G	E	W	W	H	C	C	G	P	G	C	G	W	V	V	D	A	C	7.1c

Class 1

		Y	S	F	E	G	W	G	C	I	G	P	G	C	A	Y	L	F	E	G	H	1.1.2
		Y	E	W	E	G	W	G	C	I	G	P	G	C	P	A	L	G	F	G	Y	1.1.FC
		Q	R	N	E	A	W	G	C	I	G	P	G	C	E	M	L	C	A	W	C	1.1.FA
		T	Q	A	E	R	W	G	C	I	G	P	G	C	E	C	L	M	S	C	V	1.1.FB
		C	I	D	E	T	W	G	C	I	G	P	G	C	E	E	L	R	C	K	R	1.1.FH
		N	V	C	E	F	W	G	C	I	G	P	G	C	A	Q	L	C				1.1.CF

Figure 17

Inhibition of 1.1.FI-phage Binding to HER2
by HER2 Monoclonal Antibodies

Figure 13

FACS Analysis of 1.1Fl-Zb Binding to Cells
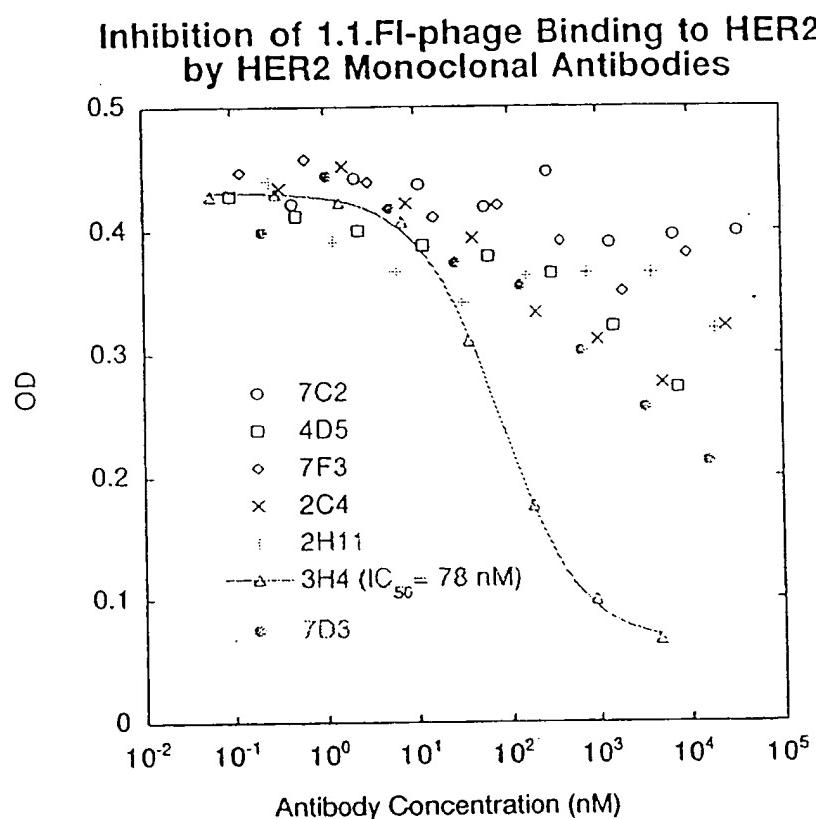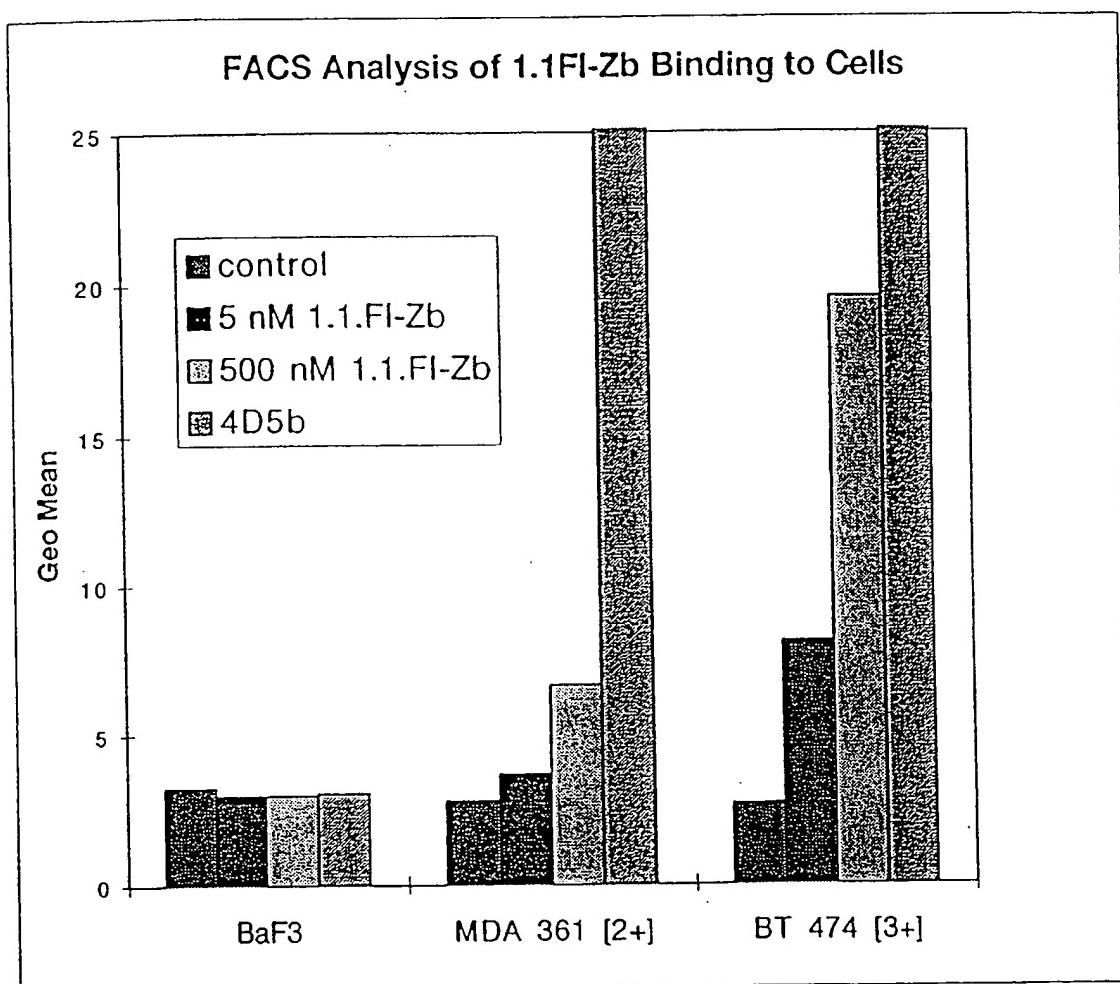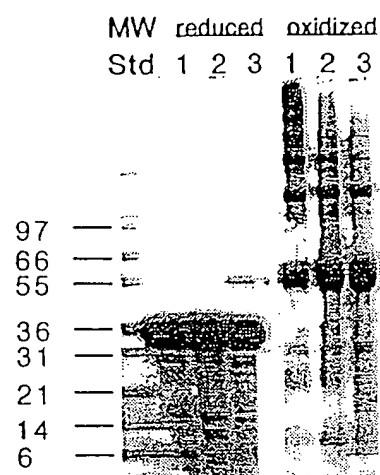
Figure 14

1 = Control Fc (no peptide fusion)
2 = HER2-Fc fusion (1.1 FI-Fc)

FIGURE 15

clone   Sequence

Pool 1

1.1   Y E V E A W D C M G P G G A N L F E A H

Library 1.1

1.1.8
1.1.9
1.1.10
1.1.1
1.1.2
1.1.6
1.1.11
1.1.3
1.1.4
1.1.5
1.1.7

Consensus   Y E F G W G C I G P C G A Y L F E A H

Pool 7

7.1   C S W V L V Q C G G E W W H C C G V G C G L V V N A C

Library 7.1

7.1.1
7.1.9
7.1.6
7.1.5
7.1.3
7.1.4
7.1.2
7.1.7
7.1.8
7.1.11

Consensus   C A W V S V E C G C E W W H C C C P G C G W V V D A C

Figure 16

Pool 1

| 1.1 | Y E V E A W D C M G P G C A N L F E A H |
| consensus | Y E F E Q W Q C I G P G C A Y L F E A H |

Library 1.1.F   - - - E - W Q C I G P G C - - L - - - -

1.1.FC	Y E W G P A G F G Y
1.1.FN	R W D E E W V V R K
1.1.FA	Q R N A E M C A W C
1.1.FM	L S P T E M C S W C
1.1.FE	E N W M K F C E P C
1.1.FH	C I D T E E R C K R
1.1.FB	T Q A R E C M S C V
1.1.FF	A P R V A C L R C I
1.1.FI	Q V Y S A C Q A C L
1.1.FK	R T E Q R C L S C L
1.1.FD	F A G S E C I G C L
1.1.FQ	T A R V N C L A C L
1.1.FS	R P H P S C L S C I
1.1.FX	A S H V K C Q A C M
1.1.FY	K L N E A C L Q C W
1.1.FZ	K L N D A C L - C -

Library 1.1.N   E - W Q C I G P G C - - L - - - -

1.1.NC	V K A C D W C
1.1.NB	R R M C E W C
1.1.NM	V D M C N W C
1.1.NQ	V S M C G W C
1.1.NA	V E C M N C L
1.1.NG	G E C L R C L
1.1.NH	G G C L K C L
1.1.NE	P A C L G C L
1.1.NJ	E A C L N C I
1.1.NF	Q K C M G C L
1.1.NK	Q G C L R C L
1.1.NR	A G C M A C L

Library 1.1.C   - - - E - W Q C I G P G C - - L

1.1.CF	N V C F A Q C
1.1.CA	H W M R G F
1.1.CB	N W P G K L
1.1.CC	F N W K R T
1.1.CD	F S G R Q V
1.1.CE	G G W G R Y
1.1.CG	L W F R T -
1.1.CH	G I P G E W
1.1.CI	W W T R S M
1.1.CJ	- C - R S M
1.1.CL	L G W R R A
1.1.CM	S P W G R W
1.1.CP	R G W G S F

Figure *17*

HER2 Competition ELISA

Sequence Listing

<110> Genentech, Inc.

<120> Compounds that Bind HER2

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<150> US 60/142,232
<151> 1999-07-02

<160> 162

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<212> PRT
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<220>
<223> synthetic peptide sequence

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 1   5   10   15

Leu Cys Ala Trp Cys
                20

<210> 2
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 2
Leu Ser Pro Glu Thr Trp Gly Cys Ile Gly Pro Gly Cys Glu Met
 1   5   10   15

Leu Cys Ser Trp Cys
                20

<210> 3
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

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Glu Asn Trp Glu Met Trp Gly Cys Ile Gly Pro Gly Cys Lys Phe
 1   5   10   15

Leu Cys Glu Pro Cys
                20

<210> 4
<211> 17
<212> PRT
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```
<220>
<223> synthetic peptide sequence

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 Glu Val Trp Gly Cys Ile Gly Pro Gly Cys Lys Ala Leu Cys Asp
  1               5                  10                  15

 Trp Cys


<210> 5
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 5
 Glu Arg Trp Gly Cys Ile Gly Pro Gly Cys Arg Met Leu Cys Glu
  1               5                  10                  15

 Trp Cys


<210> 6
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 6
 Glu Val Trp Gly Cys Ile Gly Pro Gly Cys Asp Met Leu Cys Asn
  1               5                  10                  15

 Trp Cys


<210> 7
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 7
 Glu Val Trp Gly Cys Ile Gly Pro Gly Cys Ser Met Leu Cys Gly
  1               5                  10                  15

 Trp Cys


<210> 8
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<220>
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        <221> unsure
        <222> 1-3, 5, 14, 18
        <223> unknown amino acid

        <400> 8
5       Xaa Xaa Xaa Glu Xaa Trp Gly Cys Ile Gly Pro Gly Cys Xaa Met
            1               5                   10                  15

        Leu Cys Xaa Trp Cys
                        20

        <210> 9
10      <211> 20
        <212> PRT
        <213> Artificial Sequence

        <220>
        <223> synthetic peptide sequence

15      <400> 9
        Ala Ser His Glu Val Trp Gly Cys Ile Gly Pro Gly Cys Lys Cys
            1               5                   10                  15

        Leu Gln Ala Cys Met
                        20

20      <210> 10
        <211> 20
        <212> PRT
        <213> Artificial Sequence

        <220>
25      <223> synthetic peptide sequence

        <400> 10
        Lys Leu Asn Glu Glu Trp Gly Cys Ile Gly Pro Gly Cys Ala Cys
            1               5                   10                  15

        Leu Leu Gln Cys Trp
30                          20

        <210> 11
        <211> 20
        <212> PRT
        <213> Artificial Sequence

35      <220>
        <223> synthetic peptide sequence

        <220>
        <221> unsure
        <222> 18, 20
40      <223> unknown amino acid

        <400> 11
        Lys Leu Asn Glu Asp Trp Gly Cys Ile Gly Pro Gly Cys Ala Cys
            1               5                   10                  15

        Leu Leu Xaa Cys Xaa
45                          20

        <210> 12
        <211> 20
        <212> PRT
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<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

5   <400> 12
     Thr Gln Ala Glu Arg Trp Gly Cys Ile Gly Pro Gly Cys Glu Cys
      1   5   10   15

     Leu Met Ser Cys Val
                      20

     <210> 13
10   <211> 20
     <212> PRT
     <213> Artificial Sequence

     <220>
     <223> synthetic peptide sequence

15   <400> 13
     Ala Pro Arg Glu Val Trp Gly Cys Ile Gly Pro Gly Cys Ala Cys
      1   5   10   15

     Leu Leu Arg Cys Ile
                      20

20   <210> 14
     <211> 20
     <212> PRT
     <213> Artificial Sequence

     <220>
25   <223> synthetic peptide sequence

     <400> 14
     Gln Val Tyr Glu Ser Trp Gly Cys Ile Gly Pro Gly Cys Ala Cys
      1   5   10   15

     Leu Gln Ala Cys Leu
30   20

     <210> 15
     <211> 20
     <212> PRT
     <213> Artificial Sequence

35   <220>
     <223> synthetic peptide sequence

     <400> 15
     Arg Thr Glu Glu Gln Trp Gly Cys Ile Gly Pro Gly Cys Arg Cys
      1   5   10   15

40   Leu Leu Ser Cys Leu
                      20

     <210> 16
     <211> 20
     <212> PRT
45   <213> Artificial Sequence

     <220>
     <223> synthetic peptide sequence

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      <400> 16
      Phe Ala Gly Glu Ser Trp Gly Cys Ile Gly Pro Gly Cys Glu Cys
        1           5               10              15

      Leu Ile Gly Cys Leu
5                     20


      <210> 17
      <211> 20
      <212> PRT
      <213> Artificial Sequence

10    <220>
      <223> synthetic peptide sequence

      <400> 17
      Thr Ala Arg Glu Val Trp Gly Cys Ile Gly Pro Gly Cys Asn Cys
        1           5               10              15

15    Leu Leu Ala Cys Leu
                      20


      <210> 18
      <211> 20
      <212> PRT
20    <213> Artificial Sequence

      <220>
      <223> synthetic peptide sequence

      <400> 18
      Arg Pro His Glu Pro Trp Gly Cys Ile Gly Pro Gly Cys Ser Cys
25      1           5               10              15

      Leu Leu Ser Cys Ile
                      20


      <210> 19
      <211> 17
30    <212> PRT
      <213> Artificial Sequence

      <220>
      <223> synthetic peptide sequence

      <400> 19
35    Glu Val Trp Gly Cys Ile Gly Pro Gly Cys Glu Cys Leu Met Asn
        1           5               10              15

      Cys Leu


      <210> 20
      <211> 17
40    <212> PRT
      <213> Artificial Sequence

      <220>
      <223> synthetic peptide sequence

      <400> 20
45    Glu Gly Trp Gly Cys Ile Gly Pro Gly Cys Glu Cys Leu Leu Arg
        1           5               10              15
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Cys Leu

<210> 21
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 21
Glu Gly Trp Gly Cys Ile Gly Pro Gly Cys Gly Cys Leu Leu Lys
1   5   10   15

Cys Leu

<210> 22
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 22
Glu Pro Trp Gly Cys Ile Gly Pro Gly Cys Ala Cys Leu Leu Gly
1   5   10   15

Cys Leu

<210> 23
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 23
Glu Glu Trp Gly Cys Ile Gly Pro Gly Cys Ala Cys Leu Leu Asn
1   5   10   15

Cys Ile

<210> 24
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 24
Glu Gln Trp Gly Cys Ile Gly Pro Gly Cys Lys Cys Leu Met Gly
1   5   10   15

Cys Leu

<210> 25

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        <211> 17
        <212> PRT
        <213> Artificial Sequence

        <220>
5       <223> synthetic peptide sequence

        <400> 25
        Glu Gln Trp Gly Cys Ile Gly Pro Gly Cys Gly Cys Leu Leu Arg
        1               5               10                      15

        Cys Leu


10      <210> 26
        <211> 17
        <212> PRT
        <213> Artificial Sequence

        <220>
15      <223> synthetic peptide sequence

        <400> 26
        Glu Ala Trp Gly Cys Ile Gly Pro Gly Cys Gly Cys Leu Met Ala
        1               5               10                      15

        Cys Leu


20      <210> 27
        <211> 20
        <212> PRT
        <213> Artificial Sequence

        <220>
25      <223> synthetic peptide sequence

        <220>
        <221> unsure
        <222> 1-3, 5, 14, 18
        <223> unknown amino acid

30      <400> 27
        Xaa Xaa Xaa Glu Xaa Trp Gly Cys Ile Gly Pro Gly Cys Xaa Cys
        1               5               10                      15

        Leu Leu Xaa Cys Leu
                        20

35      <210> 28
        <211> 17
        <212> PRT
        <213> Artificial Sequence

        <220>
40      <223> synthetic peptide sequence

        <400> 28
        Asn Val Cys Glu Phe Trp Gly Cys Ile Gly Pro Gly Cys Ala Gln
        1               5               10                      15

        Leu Cys
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<210> 29
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 29
Cys Ile Asp Glu Thr Trp Gly Cys Ile Gly Pro Gly Cys Glu Glu
 1               5                  10                  15

Leu Arg Cys Lys Arg
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<210> 30
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 30
Tyr Glu Trp Glu Gly Trp Gly Cys Ile Gly Pro Gly Cys Pro Ala
 1               5                  10                  15

Leu Gly Phe Gly Tyr
                20


<210> 31
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 31
Arg Trp Asp Glu Glu Trp Gly Cys Ile Gly Pro Gly Cys Glu Trp
 1               5                  10                  15

Leu Val Val Arg Lys
                20


<210> 32
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 32
His Trp Met Glu Arg Trp Gly Cys Ile Gly Pro Gly Cys Gly Phe
 1               5                  10                  15

Leu


<210> 33
<211> 16
<212> PRT
<213> Artificial Sequence
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<220>
<223> synthetic peptide sequence

<400> 33
 Asn Trp Pro Glu Gly Trp Gly Cys Ile Gly Pro Gly Cys Lys Leu
   1               5                  10                  15

   Leu


<210> 34
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 34
 Phe Asn Trp Glu Lys Trp Gly Cys Ile Gly Pro Gly Cys Arg Thr
   1               5                  10                  15

   Leu


<210> 35
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 35
 Phe Ser Gly Glu Arg Trp Gly Cys Ile Gly Pro Gly Cys Gln Val
   1               5                  10                  15

   Leu


<210> 36
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 36
 Gly Gly Trp Glu Gly Trp Gly Cys Ile Gly Pro Gly Cys Arg Tyr
   1               5                  10                  15

   Leu


<210> 37
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<220>
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      <221> unsure
      <222> 15
      <223> unknown amino acid

      <400> 37
 5    Leu Trp Phe Glu Arg Trp Gly Cys Ile Gly Pro Gly Cys Thr Xaa
          1             5               10                  15

      Leu


      <210> 38
      <211> 16
10    <212> PRT
      <213> Artificial Sequence

      <220>
      <223> synthetic peptide sequence

      <400> 38
15    Gly Ile Pro Glu Gly Trp Gly Cys Ile Gly Pro Gly Cys Glu Trp
          1             5               10                  15

      Leu


      <210> 39
      <211> 16
20    <212> PRT
      <213> Artificial Sequence

      <220>
      <223> synthetic peptide sequence

      <400> 39
25    Trp Trp Thr Glu Arg Trp Gly Cys Ile Gly Pro Gly Cys Ser Met
          1             5               10                  15

      Leu


      <210> 40
      <211> 20
30    <212> PRT
      <213> Artificial Sequence

      <220>
      <223> synthetic peptide sequence

      <220>
35    <221> unsure
      <222> 1, 3, 17-20
      <223> unknown amino acid

      <400> 40
      Xaa Cys Xaa Glu Arg Trp Gly Cys Ile Gly Pro Gly Cys Ser Met
40        1             5               10                  15

      Leu Xaa Xaa Xaa Xaa
                      20

      <210> 41
      <211> 16
45    <212> PRT
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        <213> Artificial Sequence

        <220>
        <223> synthetic peptide sequence

        <400> 41
5       Leu Gly Trp Glu Arg Trp Gly Cys Ile Gly Pro Gly Cys Arg Ala
          1               5                   10                  15

          Leu


        <210> 42
        <211> 16
10      <212> PRT
        <213> Artificial Sequence

        <220>
        <223> synthetic peptide sequence

        <400> 42
15      Ser Pro Trp Glu Gly Trp Gly Cys Ile Gly Pro Gly Cys Arg Trp
          1               5                   10                  15

          Leu


        <210> 43
        <211> 16
20      <212> PRT
        <213> Artificial Sequence

        <220>
        <223> synthetic peptide sequence

        <400> 43
25      Arg Gly Trp Glu Gly Trp Gly Cys Ile Gly Pro Gly Cys Ser Phe
          1               5                   10                  15

          Leu


        <210> 44
        <211> 20
30      <212> PRT
        <213> Artificial Sequence

        <220>
        <223> synthetic peptide sequence

        <220>
35      <221> unsure
        <222> 1-2, 5, 14-15, 17-20
        <223> unknown amino acid

        <400> 44
        Xaa Xaa Trp Glu Xaa Trp Gly Cys Ile Gly Pro Gly Cys Xaa Xaa
40        1               5                   10                  15

          Leu Xaa Xaa Xaa Xaa
                          20

        <210> 45
        <211> 27
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<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 45
Cys Ser Trp Val Leu Val Gln Cys Gly Gly Glu Trp Trp His Cys
 1               5               10                  15

Cys Gly Leu Gly Cys Gly Leu Val Val Asn Ala Cys
                20                  25


<210> 46
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 46
Cys Ala Trp Val Ser Phe Glu Cys Gly Gly Glu Val Trp His Cys
 1               5               10                  15

Cys Gly Leu Gly Cys Gly Trp Val Trp Lys Ala Cys
                20                  25


<210> 47
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 47
Cys Ala Trp Val Leu Val Gln Cys Gly Gly Glu Trp Trp His Cys
 1               5               10                  15

Cys Gly Pro Gly Cys Glu Phe Val Val Asp Ala Cys
                20                  25


<210> 48
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 48
Cys Ala Trp Val Ala Val Tyr Cys Gly Gly Glu Leu Trp His Cys
 1               5               10                  15

Cys Gly Pro Gly Cys Gly Phe Val Val Asp Ser Cys
                20                  25


<210> 49
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
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<223> synthetic peptide sequence

<400> 49
Cys Ala Trp Val Arg Val Trp Cys Phe Gly Glu Trp Trp Asp Cys
 1   5   10   15

Cys Gly Leu Gly Cys Gly Trp Val Val Asn Val Cys
                20   25

<210> 50
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 50
Cys Ala Trp Val Arg Val Leu Cys Gly Gly Glu Trp Trp His Cys
 1   5   10   15

Cys Gly Leu Gly Cys Gly Trp Val Val Glu Ala Cys
                20   25

<210> 51
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 51
Cys Ser Trp Val Ser Val Leu Cys Gly Gly Glu Trp Trp Gln Cys
 1   5   10   15

Cys Gly Pro Gly Cys Gly Leu Val Val Asn Ala Cys
                20   25

<210> 52
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 52
Cys Ser Trp Val Ser Leu Gln Cys Gly Gly Glu Trp Trp His Cys
 1   5   10   15

Cys Gly Gly Gly Cys Gly Trp Val Val Asn Val Cys
                20   25

<210> 53
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 53
Cys Ser Trp Val Leu Leu His Cys Gly Gly Glu Trp Trp His Cys

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              1             5              10              15

        Cys Gly Gly Gly Cys Gly Trp Val Gly Glu Ala Cys
                          20              25


        <210> 54
 5      <211> 27
        <212> PRT
        <213> Artificial Sequence

        <220>
        <223> synthetic peptide sequence

10      <400> 54
        Cys Ser Trp Val Leu Leu Glu Cys Gly Gly Glu Leu Trp Glu Cys
          1             5              10              15

        Cys Gly Leu Gly Cys Gly Trp Val Ala Asp Ala Cys
                          20              25

15      <210> 55
        <211> 27
        <212> PRT
        <213> Artificial Sequence

        <220>
20      <223> synthetic peptide sequence

        <400> 55
        Cys Ser Trp Val Val Phe Glu Cys Leu Gly Glu Ser Trp His Cys
          1             5              10              15

        Cys Gly Gly Gly Cys Gly Trp Val Val His Ala Cys
25                        20              25


        <210> 56
        <211> 27
        <212> PRT
        <213> Artificial Sequence

30      <220>
        <223> synthetic peptide sequence

        <400> 56
        Cys Ala Trp Val Ser Val Glu Cys Gly Gly Glu Trp Trp His Cys
          1             5              10              15

35      Cys Gly Pro Gly Cys Gly Trp Val Val Asp Ala Cys
                          20              25


        <210> 57
        <211> 20
        <212> PRT
40      <213> Artificial Sequence

        <220>
        <223> synthetic peptide sequence

        <400> 57
        Tyr Glu Val Glu Ala Trp Asp Cys Met Gly Pro Gly Cys Ala Asn
45        1             5              10              15

        Leu Phe Glu Ala His
                          20
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    <210> 58
    <211> 20
    <212> PRT
    <213> Artificial Sequence

5   <220>
    <223> synthetic peptide sequence

    <400> 58
    Tyr Glu Phe Glu Gly Trp Asp Cys Met Gly Pro Gly Cys Ala Ser
     1               5                  10                  15

10  Val Phe Gly Ala His
                    20


    <210> 59
    <211> 20
    <212> PRT
15  <213> Artificial Sequence

    <220>
    <223> synthetic peptide sequence

    <400> 59
    Tyr Glu Gly Glu Ser Trp Gly Cys Ile Gly Pro Gly Cys Ala Ser
20   1               5                  10                  15

    Leu Phe Asp Ala His
                    20

    <210> 60
    <211> 20
25  <212> PRT
    <213> Artificial Sequence

    <220>
    <223> synthetic peptide sequence

    <400> 60
30  Tyr Glu Val Glu Val Trp Glu Cys Ile Gly Pro Gly Cys Gly Tyr
     1               5                  10                  15

    Leu Phe Gly Ala His
                    20

    <210> 61
35  <211> 20
    <212> PRT
    <213> Artificial Sequence

    <220>
    <223> synthetic peptide sequence

40  <400> 61
    Tyr Glu Val Glu Gly Trp Gly Cys Met Gly Pro Gly Cys Ala Phe
     1               5                  10                  15

    Leu Leu Glu Ala His
                    20

45  <210> 62
    <211> 20
    <212> PRT
    <213> Artificial Sequence
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<220>
<223> synthetic peptide sequence

<400> 62
 Tyr Ser Phe Glu Gly Trp Gly Cys Ile Gly Pro Gly Cys Ala Tyr
  1           5               10                  15

 Leu Phe Glu Gly His
              20


<210> 63
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 63
 Tyr Asp Phe Glu Gly Trp Gly Cys Ile Gly Pro Gly Cys Gly Asn
  1           5               10                  15

 Leu Leu Glu Ala His
              20


<210> 64
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 64
 Tyr Asp Phe Glu Gly Trp Asp Cys Thr Gly Pro Gly Cys Ala Tyr
  1           5               10                  15

 Leu Phe Glu Gly His
              20


<210> 65
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 65
 Asp Glu Val Glu Ser Trp Gly Cys Ile Gly Pro Gly Cys Ala Tyr
  1           5               10                  15

 Leu Phe Gly Ala Leu
              20


<210> 66
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 66
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            Ser Glu Val Glu Val Trp His Cys Ile Gly Pro Gly Cys Val Tyr
             1               5               10               15

            Leu Phe Glu Ala Tyr
                           20

   5    <210> 67
        <211> 20
        <212> PRT
        <213> Artificial Sequence

        <220>
  10    <223> synthetic peptide sequence

        <400> 67
            Phe Glu Phe Glu Gly Trp Glu Cys Met Gly Pro Gly Cys Ala Glu
             1               5               10               15

            Leu Phe Ala Gly His
  15                       20

        <210> 68
        <211> 20
        <212> PRT
        <213> Artificial Sequence

  20    <220>
        <223> synthetic peptide sequence

        <400> 68
            His Asp Val Glu Gly Trp Gly Cys Ile Gly Pro Gly Cys Ala Asp
             1               5               10               15

  25      Leu Phe Glu Ala Phe
                           20

        <210> 69
        <211> 20
        <212> PRT
  30    <213> Artificial Sequence

        <220>
        <223> synthetic peptide sequence

        <400> 69
            Tyr Glu Phe Glu Gly Trp Gly Cys Ile Gly Pro Gly Cys Ala Tyr
  35       1               5               10               15

            Leu Phe Glu Ala His
                           20

        <210> 70
        <211> 27
  40    <212> PRT
        <213> Artificial Sequence

        <220>
        <223> synthetic peptide sequence

        <220>
  45    <221> Mutagen
        <222> 2-7, 9-14, 17-19, 21-26
        <223> More than one possible amino acid
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<400> 70
Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys
 1               5                  10                    15

Cys Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys
                20                  25

<210> 71
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<220>
<221> Mutagen
<222> 2
<223> Xaa can be Cys or Ile

<400> 71
Cys Xaa Gly Pro Gly Cys
 1               5

<210> 72
<211> 218
<212> PRT
<213> Homo sapiens

<400> 72
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
 1               5                  10                    15

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
                20                  25                    30

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
                35                  40                    45

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
                50                  55                    60

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
                65                  70                    75

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
                80                  85                    90

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                95                  100                   105

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
                110                 115                   120

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
                125                 130                   135

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
                140                 145                   150

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
                155                 160                   165

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
```

```
                      170                    175                    180

        Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
                     185                    190                    195

        Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
                     200                    205                    210
5
        Ser Leu Ser Leu Ser Pro Gly Lys
                     215


        <210> 73
        <211> 218
10      <212> PRT
        <213> Homo sapiens


        <400> 73
        Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
          1               5                  10                  15

15      Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
                      20                  25                  30

        Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
                      35                  40                  45

        Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
20                    50                  55                  60

        Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
                      65                  70                  75

        Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
                      80                  85                  90

25      Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                      95                 100                 105

        Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
                     110                 115                 120

        Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
30                   125                 130                 135

        Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
                     140                 145                 150

        Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
                     155                 160                 165

35      Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
                     170                 175                 180

        Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
                     185                 190                 195

        Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
40                   200                 205                 210

        Ser Leu Ser Leu Ser Pro Gly Lys
                     215


        <210> 74
        <211> 217
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<210> 75
<212> PRT
<213> Homo sapiens

<400> 74

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     Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro
5      1               5                  10                  15

     Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
                    20                  25                  30

     Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe
                    35                  40                  45

10   Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
                    50                  55                  60

     Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val
                    65                  70                  75

     Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
15                  80                  85                  90

     Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr
                    95                  100                 105

     Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
                    110                 115                 120

20   Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
                    125                 130                 135

     Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
                    140                 145                 150

     Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
25                  155                 160                 165

     Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
                    170                 175                 180

     Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
                    185                 190                 195

30   Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
                    200                 205                 210

     Leu Ser Leu Ser Pro Gly Lys
                    215
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<210> 75
35 <211> 218
<212> PRT
<213> Homo sapiens

<400> 75

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     Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
40     1               5                  10                  15

     Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
                    20                  25                  30

     Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln
                    35                  40                  45
```

```
        Phe Lys Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
                     50                  55                  60

        Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser
                     65                  70                  75

  5     Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
                     80                  85                  90

        Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                     95                 100                 105

        Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
 10                 110                 115                 120

        Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
                    125                 130                 135

        Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
                    140                 145                 150

 15     Glu Trp Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr Asn Thr Thr
                    155                 160                 165

        Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
                    170                 175                 180

        Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser
 20                 185                 190                 195

        Cys Ser Val Met His Glu Ala Leu His Asn Arg Phe Thr Gln Lys
                    200                 205                 210

        Ser Leu Ser Leu Ser Pro Gly Lys
                    215

 25     <210> 76
        <211> 218
        <212> PRT
        <213> Homo sapiens

        <400> 76
 30     Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
          1             5                  10                  15

        Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
                     20                  25                  30

        Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln
 35                  35                  40                  45

        Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
                     50                  55                  60

        Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
                     65                  70                  75

 40     Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
                     80                  85                  90

        Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys
                     95                 100                 105

        Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
```

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                        110   115   120

        Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser
                         125   130   135

        Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
  5   140   145   150

        Glu Trp Glx Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
                         155   160   165

        Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg
                         170   175   180

  10   Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
                         185   190   195

        Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
                         200   205   210

        Ser Leu Ser Leu Ser Leu Gly Lys
  15   215

        <210> 77
        <211> 215
        <212> PRT
        <213> Mus musculus

  20   <400> 77
        Thr Val Pro Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro
         1   5   10   15

        Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val
                          20   25   30

  25   Val Val Asp Ile Ser Lys Asp Asp Pro Glu Val Gln Phe Ser Trp
                          35   40   45

        Phe Val Asp Asp Val Glu Val His Thr Ala Gln Thr Gln Pro Arg
                          50   55   60

        Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser Glu Leu Pro
  30   65   70   75

        Ile Met His Gln Asp Cys Leu Asn Gly Lys Glu Phe Lys Cys Arg
                          80   85   90

        Val Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile Ser
                          95   100   105

  35   Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro
                         110   115   120

        Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys
                         125   130   135

        Met Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln
  40   140   145   150

        Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile
                         155   160   165

        Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val
                         170   175   180

Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val
                185   190   195

Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser Leu Ser
                200   205   210

His Ser Pro Gly Lys
                215

<210> 78
<211> 218
<212> PRT
<213> Mus musculus

<400> 78
Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro
  1   5   10   15

Pro Lys Ile Lys Asp Val Leu Met Ile Ser Leu Ser Pro Ile Val
                20   25   30

Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln
                35   40   45

Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr Ala Gln Thr
                50   55   60

Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val Val Ser
                65   70   75

Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu Phe
                80   85   90

Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg
                95   100   105

Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr
                110   115   120

Val Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr
                125   130   135

Leu Thr Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val
                140   145   150

Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr
                155   160   165

Glu Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys
                170   175   180

Leu Arg Val Glu Lys Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser
                185   190   195

Cys Ser Val Val His Glu Gly Leu His Asn His His Thr Thr Lys
                200   205   210

Ser Phe Ser Arg Thr Pro Gly Lys
                215

<210> 79
<211> 218
<212> PRT
<213> Mus musculus

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     <400> 79
        Pro Ala Pro Asn Leu Glu Gly Gly Pro Ser Val Phe Ile Phe Pro
          1               5                  10                  15

 5      Pro Asn Ile Lys Asp Val Leu Met Ile Ser Leu Thr Pro Lys Val
                         20                  25                  30

        Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln
                         35                  40                  45

        Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr Ala Gln Thr
                         50                  55                  60

10      Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Ile Arg Val Val Ser
                         65                  70                  75

        His Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu Phe
                         80                  85                  90

        Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ser Pro Ile Glu Arg
15                       95                 100                 105

        Thr Ile Ser Lys Pro Lys Gly Leu Val Arg Ala Pro Gln Val Tyr
                        110                 115                 120

        Thr Leu Pro Pro Pro Ala Glu Gln Leu Ser Arg Lys Asp Val Ser
                        125                 130                 135

20      Leu Thr Cys Leu Val Val Gly Phe Asn Pro Gly Asp Ile Ser Val
                        140                 145                 150

        Glu Trp Thr Ser Asn Gly His Thr Glu Glu Asn Tyr Lys Asp Thr
                        155                 160                 165

        Ala Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe Ile Tyr Ser Lys
25                      170                 175                 180

        Leu Asn Met Lys Thr Ser Lys Trp Glu Lys Thr Asp Ser Phe Ser
                        185                 190                 195

        Cys Asn Val Arg His Glu Gly Leu Lys Asn Tyr Tyr Leu Lys Lys
                        200                 205                 210

30      Thr Ile Ser Arg Ser Pro Gly Lys
                        215

     <210> 80
     <211> 218
     <212> PRT
35   <213> Mus musculus

     <400> 80
        Pro Pro Gly Asn Ile Leu Gly Gly Pro Ser Val Phe Ile Phe Pro
          1               5                  10                  15

        Pro Lys Pro Lys Asp Ala Leu Met Ile Ser Leu Thr Pro Lys Val .
40                       20                  25                  30

        Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro Asp Val His
                         35                  40                  45

        Val Ser Trp Phe Val Asp Asn Lys Glu Val His Thr Ala Trp Thr
                         50                  55                  60
```

```
        Gln Pro Arg Glu Ala Gln Tyr Asn Ser Thr Phe Arg Val Val Ser
                     65              70              75

        Ala Leu Pro Ile Gln His Gln Asp Trp Met Arg Gly Lys Glu Phe
                     80              85              90

    5   Lys Cys Lys Val Asn Asn Lys Ala Leu Pro Ala Pro Ile Glu Arg
                     95             100             105

        Thr Ile Ser Lys Pro Lys Gly Arg Ala Gln Thr Pro Gln Val Tyr
                    110             115             120

        Thr Ile Pro Pro Pro Arg Glu Gln Met Ser Lys Lys Lys Val Ser
   10               125             130             135

        Leu Thr Cys Leu Val Thr Asn Phe Phe Ser Glu Ala Ile Ser Val
                    140             145             150

        Glu Trp Glu Arg Asn Gly Glu Leu Glu Gln Asp Tyr Lys Asn Thr
                    155             160             165

   15   Pro Pro Ile Leu Asp Ser Asp Gly Thr Tyr Phe Leu Tyr Ser Lys
                    170             175             180

        Leu Thr Val Asp Thr Asp Ser Trp Leu Gln Gly Glu Ile Phe Thr
                    185             190             195

        Cys Ser Val Val His Glu Ala Leu His Asn His His Thr Gln Lys
   20               200             205             210

        Asn Leu Ser Arg Ser Pro Gly Lys
                    215
```

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        <210> 81
        <211> 20
   25   <212> PRT
        <213> Artificial Sequence

        <220>
        <223> synthetic peptide sequence

        <400> 81
   30   Gln Val Tyr Glu Ser Trp Gly Cys Ile Gly Pro Gly Cys Ala Cys
          1               5              10                  15

        Leu Gln Ala Cys Leu
                     20

        <210> 82
   35   <211> 46
        <212> PRT
        <213> Artificial Sequence

        <220>
        <223> synthetic peptide sequence

   40   <400> 82
        Gln Val Tyr Glu Ser Trp Gly Cys Ile Gly Pro Gly Cys Ala Cys
          1               5              10                  15

        Leu Gln Ala Cys Leu Gly Gly Gly Ser Gly Gly Gln Val Tyr Glu
                     20              25              30

   45   Ser Trp Gly Cys Ile Gly Pro Gly Cys Ala Cys Leu Gln Ala Cys
```

                        35   40   45

        Leu

        <210> 83
        <211> 27
   5   <212> PRT
        <213> Artificial Sequence

        <220>
        <223> synthetic peptide sequence

        <400> 83
  10   Cys Ala Trp Val Ser Val Glu Cys Gly Gly Glu Trp Trp His Cys
         1   5   10   15

        Cys Gly Pro Gly Cys Gly Trp Val Val Asp Ala Cys
                        20   25

        <210> 84
  15   <211> 20
        <212> PRT
        <213> Artificial Sequence

        <220>
        <223> synthetic peptide sequence

  20   <400> 84
        Tyr Ser Phe Glu Gly Trp Gly Cys Ile Gly Pro Gly Cys Ala Tyr
          1   5   10   15

        Leu Phe Glu Gly His
                        20

  25   <210> 85
        <211> 20
        <212> PRT
        <213> Artificial Sequence

        <220>
  30   <223> synthetic peptide sequence

        <400> 85
        Tyr Glu Trp Glu Gly Trp Gly Cys Ile Gly Pro Gly Cys Pro Ala
          1   5   10   15

        Leu Gly Phe Gly Tyr
  35   20

        <210> 86
        <211> 20
        <212> PRT
        <213> Artificial Sequence

  40   <220>
        <223> synthetic peptide sequence

        <400> 86
        Gln Arg Asn Glu Ala Trp Gly Cys Ile Gly Pro Gly Cys Glu Met
          1   5   10   15

  45   Leu Cys Ala Trp Cys
                        20

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<210> 87
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 87
  Thr Gln Ala Glu Arg Trp Gly Cys Ile Gly Pro Gly Cys Glu Cys
   1               5                  10                  15

  Leu Met Ser Cys Val
                  20


<210> 88
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 88
  Cys Ile Asp Glu Thr Trp Gly Cys Ile Gly Pro Gly Cys Glu Glu
   1               5                  10                  15

  Leu Arg Cys Lys Arg
                  20

<210> 89
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 89
  Asn Val Cys Glu Phe Trp Gly Cys Ile Gly Pro Gly Cys Ala Gln
   1               5                  10                  15

  Leu Cys


<210> 90
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<220>
<221> Mutagen
<222> 1-14, 16, 21-27
<223> More than one possible amino acid

<400> 90
  Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
   1               5                  10                  15

  Xaa Gly Pro Gly Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa
                  20                  25
```

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```
<210> 91
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<220>
<221> Mutagen
<222> 1-3, 5, 14-15, 17-20
<223> More than one possible amino acid

<400> 91
 Xaa Xaa Xaa Glu Xaa Trp Gly Cys Ile Gly Pro Gly Cys Xaa Xaa
   1           5               10              15


 Leu Xaa Xaa Xaa Xaa
                 20

<210> 92
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<220>
<221> Mutagen
<222> 1-7, 9, 14-20
<223> More than one possible amino acid

<400> 92
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Gly Pro Gly Cys Xaa Xaa
   1           5               10              15


 Xaa Xaa Xaa Xaa Xaa
                 20

<210> 93
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<220>
<221> Mutagen
<222> 1-5, 7, 9, 14-20
<223> More than one possible amino acid

<400> 93
 Xaa Xaa Xaa Xaa Xaa Trp Xaa Cys Xaa Gly Pro Gly Cys Xaa Xaa
   1           5               10              15


 Xaa Xaa Xaa Xaa Xaa
                 20

<210> 94
<211> 4
<212> PRT
<213> Artificial Sequence
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        <220>
        <223> synthetic peptide sequence

        <400> 94
         Phe Gly Ala His
5          1

        <210> 95
        <211> 4
        <212> PRT
        <213> Artificial Sequence

10      <220>
        <223> synthetic peptide sequence

        <400> 95
         Phe Asp Ala His
           1

15      <210> 96
        <211> 4
        <212> PRT
        <213> Artificial Sequence

        <220>
20      <223> synthetic peptide sequence

        <400> 96
         Leu Glu Ala His
           1

        <210> 97
25      <211> 4
        <212> PRT
        <213> Artificial Sequence

        <220>
        <223> synthetic peptide sequence

30      <400> 97
         Phe Glu Gly His
           1

        <210> 98
        <211> 4
35      <212> PRT
        <213> Artificial Sequence

        <220>
        <223> synthetic peptide sequence

        <400> 98
40       Phe Gly Ala Leu
           1

        <210> 99
        <211> 4
        <212> PRT
45      <213> Artificial Sequence

        <220>
        <223> synthetic peptide sequence

        <400> 99
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     Phe Glu Ala Tyr
        1

     <210> 100
     <211> 4
 5   <212> PRT
     <213> Artificial Sequence

     <220>
     <223> synthetic peptide sequence

     <400> 100
10   Phe Ala Gly His
        1

     <210> 101
     <211> 4
     <212> PRT
15   <213> Artificial Sequence

     <220>
     <223> synthetic peptide sequence

     <400> 101
      Phe Glu Ala Phe
20        1

     <210> 102
     <211> 4
     <212> PRT
     <213> Artificial Sequence

25   <220>
     <223> synthetic peptide sequence

     <400> 102
      Gln Ala Cys Met
         1

30   <210> 103
     <211> 4
     <212> PRT
     <213> Artificial Sequence

     <220>
35   <223> synthetic peptide sequence

     <400> 103
      Leu Gln Cys Trp
         1

     <210> 104
40   <211> 4
     <212> PRT
     <213> Artificial Sequence

     <220>
     <223> synthetic peptide sequence

45   <400> 104
      Met Ser Cys Val
         1

     <210> 105
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     <211> 4
     <212> PRT
     <213> Artificial Sequence

     <220>
5    <223> synthetic peptide sequence

     <400> 105
      Leu Arg Cys Ile
        1

     <210> 106
10   <211> 4
     <212> PRT
     <213> Artificial Sequence

     <220>
     <223> synthetic peptide sequence

15   <400> 106
      Gln Ala Cys Leu
        1

     <210> 107
     <211> 4
20   <212> PRT
     <213> Artificial Sequence

     <220>
     <223> synthetic peptide sequence

     <400> 107
25    Leu Ser Cys Leu
        1

     <210> 108
     <211> 4
     <212> PRT
30   <213> Artificial Sequence

     <220>
     <223> synthetic peptide sequence

     <400> 108
      Ile Gly Cys Leu
35      1

     <210> 109
     <211> 4
     <212> PRT
     <213> Artificial Sequence

40   <220>
     <223> synthetic peptide sequence

     <400> 109
      Leu Ala Cys Leu
        1

45   <210> 110
     <211> 4
     <212> PRT
     <213> Artificial Sequence
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     <220>
     <223> synthetic peptide sequence

     <400> 110
      Leu Ser Cys Ile
5        1

     <210> 111
     <211> 4
     <212> PRT
     <213> Artificial Sequence

10   <220>
     <223> synthetic peptide sequence

     <400> 111
      Met Asn Cys Leu
         1

15   <210> 112
     <211> 4
     <212> PRT
     <213> Artificial Sequence

     <220>
20   <223> synthetic peptide sequence

     <400> 112
      Leu Arg Cys Leu
         1

     <210> 113
25   <211> 4
     <212> PRT
     <213> Artificial Sequence

     <220>
     <223> synthetic peptide sequence

30   <400> 113
      Leu Lys Cys Leu
         1

     <210> 114
     <211> 4
35   <212> PRT
     <213> Artificial Sequence

     <220>
     <223> synthetic peptide sequence

     <400> 114
40    Leu Gly Cys Leu
         1

     <210> 115
     <211> 4
     <212> PRT
45   <213> Artificial Sequence

     <220>
     <223> synthetic peptide sequence

     <400> 115
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   Leu Asn Cys Ile
      1

   <210> 116
   <211> 4
5  <212> PRT
   <213> Artificial Sequence

   <220>
   <223> synthetic peptide sequence

   <400> 116
10 Met Gly Cys Leu
      1

   <210> 117
   <211> 4
   <212> PRT
15 <213> Artificial Sequence

   <220>
   <223> synthetic peptide sequence

   <400> 117
   Met Ala Cys Leu
20    1

   <210> 118
   <211> 4
   <212> PRT
   <213> Artificial Sequence

25 <220>
   <223> synthetic peptide sequence

   <400> 118
   Cys Ala Trp Cys
      1

30 <210> 119
   <211> 4
   <212> PRT
   <213> Artificial Sequence

   <220>
35 <223> synthetic peptide sequence

   <400> 119
   Cys Ser Trp Cys
      1

   <210> 120
40 <211> 4
   <212> PRT
   <213> Artificial Sequence

   <220>
   <223> synthetic peptide sequence

45 <400> 120
   Cys Glu Pro Cys
      1

   <210> 121
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```
        <211> 4
        <212> PRT
        <213> Artificial Sequence

        <220>
5       <223> synthetic peptide sequence

        <400> 121
         Cys Asp Trp Cys
           1

        <210> 122
10      <211> 4
        <212> PRT
        <213> Artificial Sequence

        <220>
        <223> synthetic peptide sequence

15      <400> 122
         Cys Glu Trp Cys
           1

        <210> 123
        <211> 4
20      <212> PRT
        <213> Artificial Sequence

        <220>
        <223> synthetic peptide sequence

        <400> 123
25       Cys Asn Trp Cys
           1

        <210> 124
        <211> 4
        <212> PRT
30      <213> Artificial Sequence

        <220>
        <223> synthetic peptide sequence

        <400> 124
         Cys Gly Trp Cys
35         1

        <210> 125
        <211> 27
        <212> PRT
        <213> Artificial Sequence

40      <220>
        <223> synthetic peptide sequence

        <220>
        <221> Mutagen
        <222> 2-7, 9-14, 17-19, 21-26
45      <223> More than one possible amino acid

        <400> 125
         Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys
           1               5                  10                  15
```

```
        Cys Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys
                        20                      25
```

        <210> 126
        <211> 27
5   <212> PRT
        <213> Artificial Sequence

        <220>
        <223> synthetic peptide sequence

        <220>
10   <221> Mutagen
        <222> 1-10, 12, 14, 21-27
        <223> More than one possible amino acid

        <400> 126
```
         Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Glu Xaa Trp Xaa Cys
15        1               5                   10                  15
```

```
         Cys Gly Pro Gly Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa
                         20                      25
```

        <210> 127
        <211> 10
20   <212> PRT
        <213> Artificial Sequence

        <220>
        <223> synthetic peptide sequence

        <220>
25   <221> Mutagen
        <222> 2, 5-7, 9
        <223> More than one possible amino acid

        <400> 127
```
         Cys Xaa Trp Val Xaa Xaa Xaa Cys Xaa Gly
30        1               5                   10
```

        <210> 128
        <211> 10
        <212> PRT
        <213> Artificial Sequence

35   <220>
        <223> synthetic peptide sequence

        <220>
        <221> Mutagen
        <222> 6-7
40   <223> More than one possible amino acid

        <400> 128
```
         Cys Ala Trp Val Leu Xaa Xaa Cys Gly Gly
          1               5                   10
```

        <210> 129
45   <211> 6
        <212> PRT
        <213> Artificial Sequence

        <220>
        <223> synthetic peptide sequence

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       <400> 129
        Gly Gly Gly Ser Gly Gly
          1               5


       <210> 130
  5    <211> 6
       <212> PRT
       <213> Artificial Sequence

       <220>
       <223> synthetic peptide sequence

 10    <400> 130
        Gly Gly Gly Ser Ser Gly
          1               5


       <210> 131
       <211> 6
 15    <212> PRT
       <213> Artificial Sequence

       <220>
       <223> synthetic peptide sequence

       <400> 131
 20     Gly Gly Gly Arg Gly Gly
          1               5


       <210> 132
       <211> 20
       <212> PRT
 25    <213> Artificial Sequence

       <220>
       <223> synthetic peptide sequence

       <400> 132
        Tyr Glu Val Glu Ala Trp Asp Cys Met Gly Pro Gly Cys Ala Asn
 30       1               5                  10                  15

        Leu Phe Glu Ala His
                      20

       <210> 133
       <211> 20
 35    <212> PRT
       <213> Artificial Sequence

       <220>
       <223> synthetic peptide sequence

       <400> 133
 40     Ser Ser Glu Cys Ala Cys Asp Lys Gly Gly Arg Arg Val Leu Cys
          1               5                  10                  15

        Ile Asn Lys Val Gly
                      20

       <210> 134
 45    <211> 20
       <212> PRT
       <213> Artificial Sequence

       <220>
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<223> synthetic peptide sequence

<400> 134
Glu Pro His Gly Cys Ser Leu Trp Asp Trp Glu Leu Arg Thr Cys
 1   5   10   15

5   Ser Glu Tyr Ala Asn
                         20

<210> 135
<211> 20
<212> PRT
10   <213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 135
       Lys Glu Arg Pro Cys Ala Gly Asp Ala Pro Arg Lys Gly Val Cys
15   1   5   10   15

       His Val Ala Thr His
                         20

<210> 136
<211> 20
20   <212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

<400> 136
25   Lys Val Arg Ser Cys Ile Glu Glu Ser Leu Asp Thr Arg Arg Cys
        1   5   10   15

       Tyr Leu Val Val Glu
                         20

<210> 137
30   <211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence

35   <400> 137
       Ala Lys Thr Ser Ser Cys Gly Glu His Glu Glu Arg Arg Ala Val
        1   5   10   15

       Cys Val Leu Ser Arg
                         20

40   <210> 138
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
45   <223> synthetic peptide sequence

<400> 138
       Lys Val Trp Ser Val Gln Ser Pro

```
            1                   5

     <210> 139
     <211> 8
     <212> PRT
5    <213> Artificial Sequence

     <220>
     <223> synthetic peptide sequence

     <400> 139
       Gly Lys Val Gln Arg Cys Ile Pro
10        1               5

     <210> 140
     <211> 10
     <212> PRT
     <213> Artificial Sequence

15   <220>
     <223> synthetic peptide sequence

     <400> 140
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20   <210> 141
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25         1               5                   10


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 Thr

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Ser Ala Cys Cys

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Cys Gly Leu Gly Cys Gly Leu Val Val Asn Ala Cys
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<210> 158
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<400> 158
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       cctgcaggcc tgcctgggag gcgggagctc cggc 84

10    <210> 160
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       cgatgcatcc ccaggactcg tacacctggg 80

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